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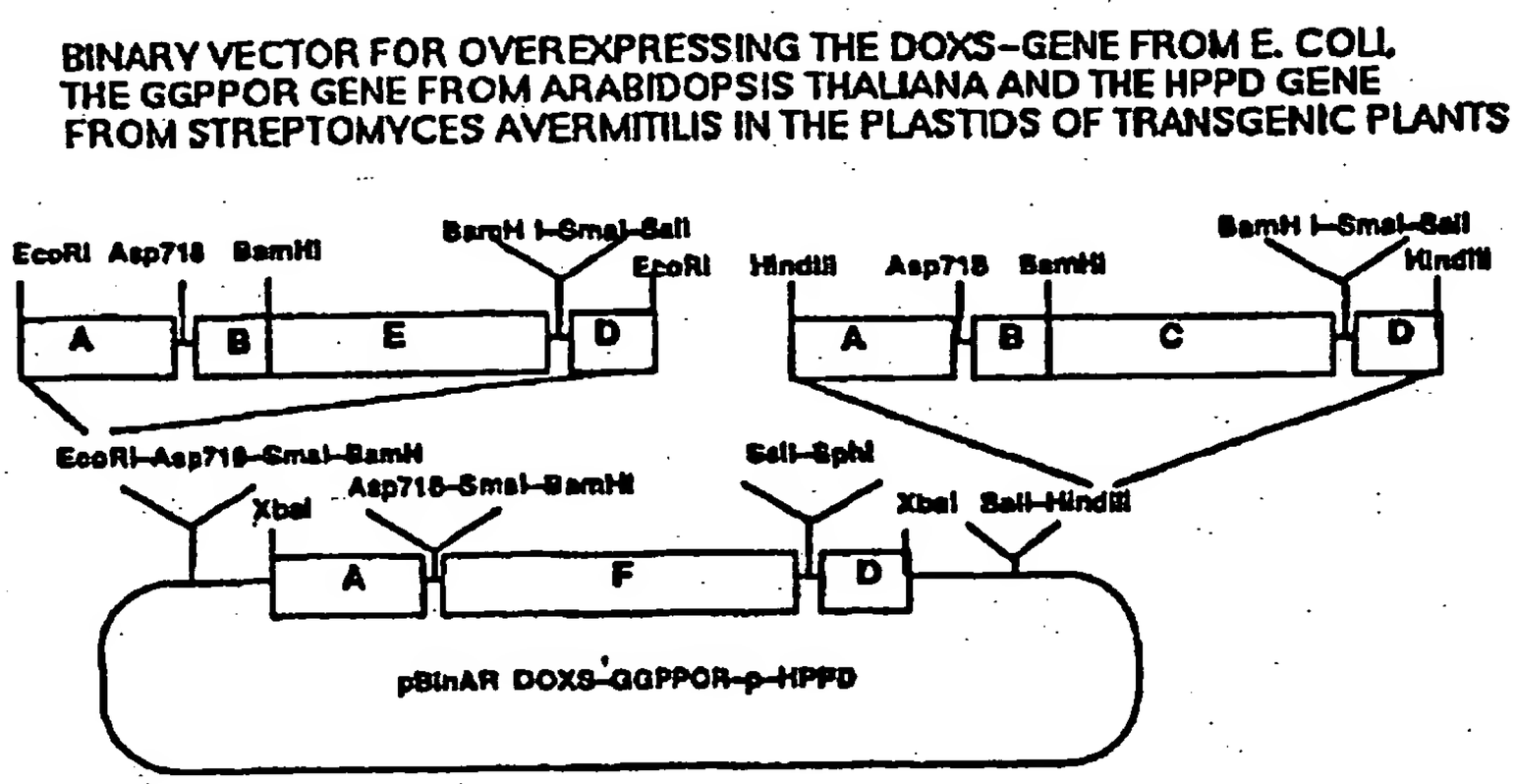
(54) SEQUENCE ADN CODANT POUR UNE 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE ET SA SURPRODUCTION DANS LES PLANTES

(54) DNA SEQUENCE CODING FOR A 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE AND THE OVERPRODUCTION THEREOF IN PLANTS



(57) Method for the production of plants with enhanced vitamin E biosynthesis efficiency by overproduction of a 1-deoxy-D-xylulose-5-phosphate synthase gene from Arabidopsis or E. coli.

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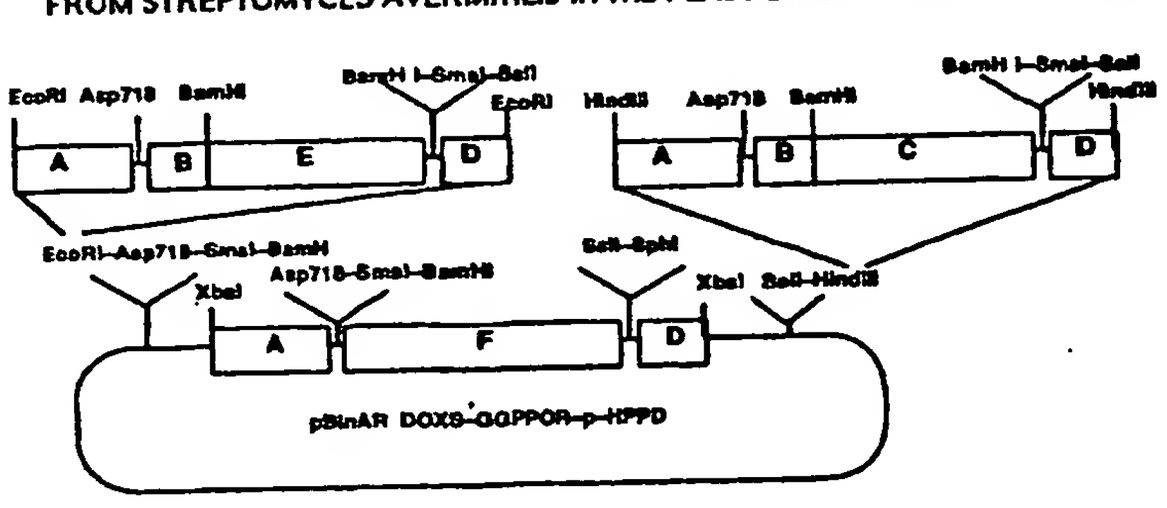
(54) Title: DNA SEQUENCE CODING FOR A 1–DEOXY–D–XYLULOSE–5–PHOSPHATE SYNTHASE AND THE OVERPRODUCTION THEREOF IN PLANTS

(54) Bezeichnung: DNA–SEQUENZ KODIEREND FÜR EINE 1–DEOXY–D–XYLULOSE–5–PHOSPHAT SYNTHASE UND DEREN ÜBERPRODUKTION IN PFLANZEN

Binärer Vektor zur Überexpression des DOXS-Gens aus E. coli, des GGPPOR-Gens aus *Arabidopsis thaliana* und des HPPD-Gens aus *Streptomyces avermitilis* in den Plastiden transgener Pflanzen.

BINARY VECTOR FOR OVEREXPRESSING THE DOXS–GENE FROM E. COLI, THE GGPPOR GENE FROM ARABIDOPSIS THALIANA AND THE HPPD GENE FROM STREPTOMYCES AVERMITILIS IN THE PLASTIDS OF TRANSGENIC PLANTS



(57) Abstract

Method for the production of plants with enhanced vitamin E biosynthesis efficiency by overproduction of a 1–deoxy–D–xylulose–5–phosphate synthase gene from Arabidopsis or E. coli.

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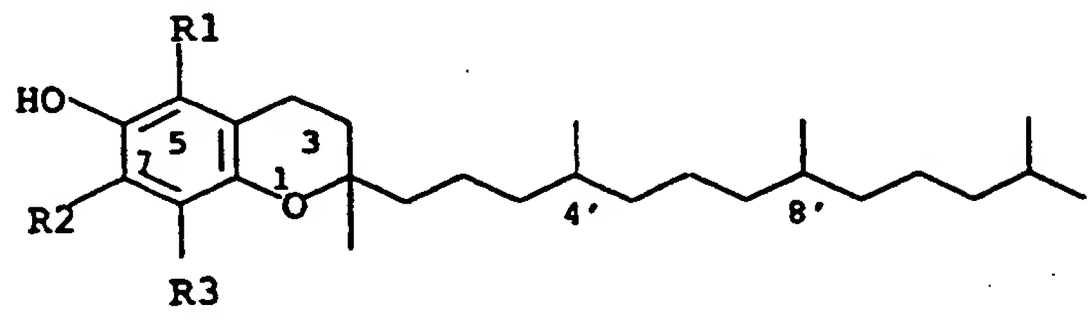
DNA SEQUENCE CODING FOR A 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE AND THE OVERPRODUCTION THEREOF IN PLANTS

The present invention relates to the use of DNA sequences coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents, specifically to the use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 or of a DNA sequence hybridizing with the latter, to the use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and of a DNA sequence SEQ ID No. 5 or DNA sequences hybridizing with the latter and coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and a p-dydroxyphenylpyruvate dioxygenase (HPPD) for producing plants with increased content of tocopherols, vitamin K, chlorophylls and/or carotenoids, to the use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and of a DNA sequence SEQ ID No. 7 or DNA sequences hybridizing with the latter and coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) for producing plants with increased content of tocopherols, vitamin K, chlorophylls and/or carotenoids, to the use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3, of a DNA sequence SEQ ID No. 5 and of a DNA sequence SEQ ID No. 7 or DNA sequences hybridizing with the latter and coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS), a hydroxyphenylpyruvate dioxygenase (HPPD) and a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) for producing plants with increased content of tocopherols, vitamin K, chlorophylls and/or carotenoids, to processes for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents, comprising a DNA sequence SEQ-ID No. 1 or SEQ ID No. 3; SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5; SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 7 or a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5 and SEQ ID No. 7, to the plants themselves produced in this way, and to the use of SEQ ID No. 1 or SEQ ID No. 3 for producing a test system for identifying DOXS inhibitors.

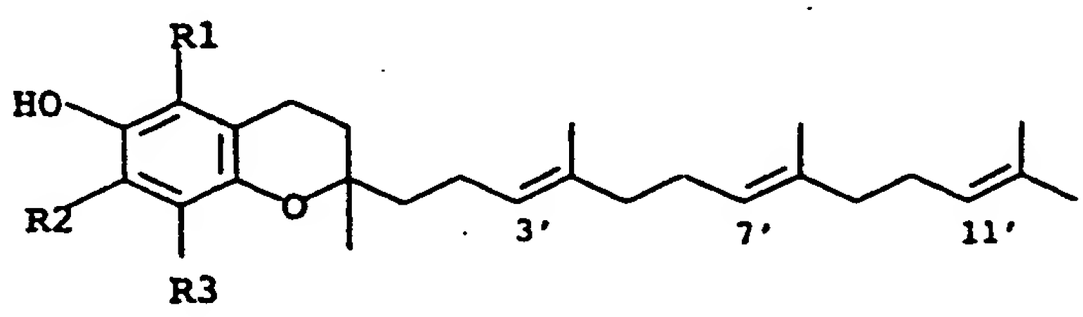
An important aim of molecular genetic work on plants to date has been the generation of plants with increased content of sugars, enzymes and amino acids. However, there is also commercial interest in the development of plants with increased content of vitamins, such as increasing the tocopherol content.

The eight compounds with vitamin E activity which occur in nature are derivatives of 6-chromanol (Ullmann's Encyclopedia of Industrial Chemistry, Vol. A 27 (1996), VCH Verlagsgesellschaft,

Chapter 4, 478–488, Vitamin E). The first group (1a-d) is derived from tocopherol, while the second group consists of derivatives of tocotrienol (2a- d):



1a, α–Tocopherol: $R^1 = R^2 = R^3 = CH_3$
1b, β–Tocopherol [148–03–8]: $R^1 = R^3 = CH_3$, $R^2 = H$
1c, γ–Tocopherol [54–28–4]: $R^1 = H$, $R^2 = R^3 = CH_3$
1d, δ–Tocopherol [119–13–1]: $R^1 = R^2 = H$, $R^3 = CH_3$



2a, α–Tocotrienol [1721–51–3]: $R^1 = R^2 = R^3 = CH_3$
2b, β–Tocotrienol [490–23–3]: $R^1 = R^3 = CH_3$, $R^2 = H$
2c, γ–Tocotrienol [14101–61–2]: $R^1 = H$, $R^2 = R^3 = CH_3$
2d, δ–Tocotrienol [25612–59–3]: $R^1 = R^2 = H$, $R^3 = CH_3$

The compound of great commercial importance is α–tocopherol.

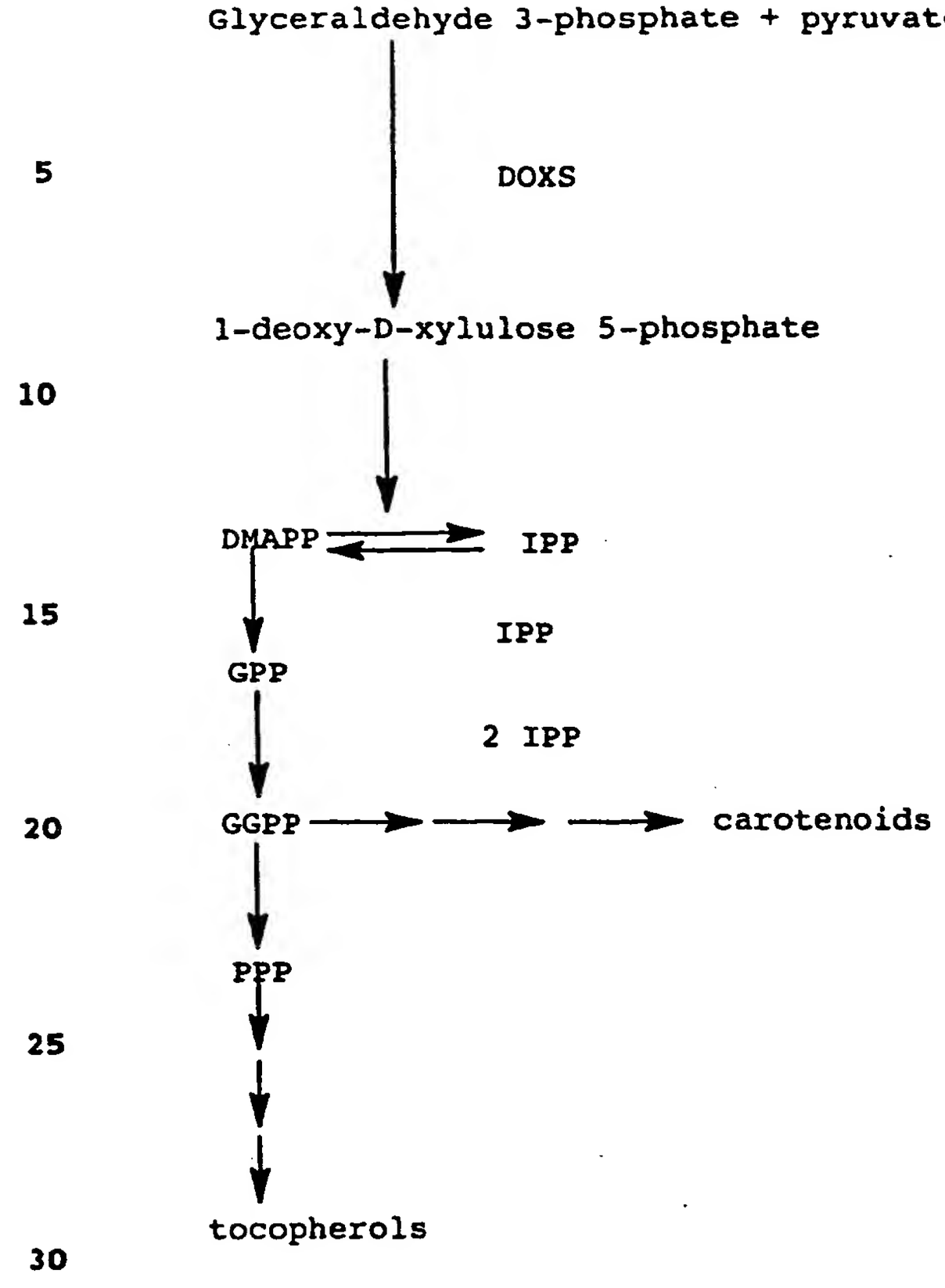
There are limits on the development of crop plants with increased tocopherol content through tissue culture or seed mutagenesis and natural selection. Thus, on the one hand, the tocopherol content must be measurable even in the tissue culture and, on the other hand, the only plants which can be manipulated by tissue culture techniques are those which can be regenerated to whole plants from cell cultures. In addition, crop plants may, after mutagenesis and selection, show unwanted properties which must be eliminated again by backcrossings, several times in some instances. Moreover increasing the tocopherol content by crossing would be retricted to plants of the same species.

For these reasons, the genetic engineering procedure of isolating, and transferring into target crop plants, an essential biosynthesis gene coding for tocopherol synthesis activity is

superior to the classical breeding method. The preconditions for this process are that the biosynthesis and its regulation are known and that genes which influence the biosynthetic activity are identified.

Isoprenoids or terpenoids consist of various classes of lipid-soluble molecules and are formed partly or completely of C_5-isoprene units. Pure prenyl lipids (e.g. carotenoids) consist of C skeletons derived exclusively from isoprene units, whereas mixed prenyl lipids (e.g. chlorophyll) have an isoprenoid side chain connected to an aromatic nucleus.

The biosynthesis of prenyl lipids starts from 3 x acetyl-CoA units, which are converted via ß-hydroxymethylglutaryl-CoA (HMG-CoA) and mevalonate into the initial isoprene unit (C_5), isopentenyl pyrophosphate (IPP). It has recently been shown by in vivo feeding experiments with C^{13} that a mevalonate-independent pathway is followed in various eubacteria, green algae and plant chloroplasts to produce IPP:



This entails hydroxyethylthiamine, which is produced by decarboxylation of pyruvate, and glyceraldehyde 3-phosphate (3-GAP) being converted, in a "transketolase" reaction mediated by 1-deoxy-D-xylulose-5-phosphate synthase, initially into 1-deoxy-D-xylulose-5-phosphate (Schwender et al., FEBS Lett. 414(1),129-134(1997); Arigoni et al., Proc.Natl.Acad.Sci USA 94(2), 10600-10605 (1997); Lange et al., Proc.Natl.Acad.Sci.USA 95(5), 2100-2104(1998); Lichtenthaler et al., FEBS Lett. 400(3), 271-274(1997). The latter is then converted by an intramolecular rearrangement into IPP (Arigoni et al., 1997). Biochemical data indicate that the mevalonate pathway operates in the cytosol and leads to the production of phytosterols. The antibiotic mevinolin, a specific inhibitor of mevalonate production, leads only to inhibition of sterol biosynthesis in the cytoplasm, whereas prenyl lipid production in the plastids is unaffected (Bach and Lichtenthaler, Physiol. Plant 59(1983), 50-60. By

contrast, the mevalonate-independent pathway has a plastidic localization and leads mainly to the production of carotenoids and plastidic prenyl lipids (Schwender et al., 1997; Arigoni et al, 1997).

IPP is in equilibrium with its isomer, dimethylallyl pyrophosphate (DMAPP). Condensation of IPP with DMAPP in a head-tail addition affords the monoterpene (C_{10}) geranyl pyrophosphate (GPP). Addition of further IPP units results in the sesquiterpene (C_{15}) farnesy pyrophosphate (FPP) and the diterpene (C_{20}) geranyl-geranyl pyrophosphate (GGPP). Linkage of two GGPP molecules results in the production of the C_{40} precursors for carotenoids. GGPP is transformed by a prenyl chain hydrogenase into phytyl pyrophosphate (PPP), the starting material for further production of tocopherols.

The ring structures of the mixed prenyl lipids which lead to the production of vitamins E and K comprise quinones whose initial metabolites are derived from the shikimate pathway. The aromatic amino acids phenylalanine and tyrosine are converted into hydroxyphenylpyruvate, which is transformed by dioxygenation into homogentisic acid. The latter is bound to PPP in order to produce 2-methyl-6-phytylquinol, the precursor of α-tocopherol and α-tocoquinone. Methylation steps with S-adenosylmethionine as methyl group donor result initially in 2,3-dimethyl-6-phytyl-quinol and then, by cyclization, in γ-tocopherol and, by methylation again, in α-tocopherol (Richter, Biochemie der Pflanzen, Georg Thieme Verlag Stuttgart, 1996).

Examples are to be found in the literature showing that manipulation of an enzyme can influence the direction of the metabolyte flux. It was possible in experiments with modified expression of phytoene synthase, which links two GGPP molecules together to give 15-cis-phytoene, to measure a direct effect on the amounts of carotenoids in these transgenic tomato plants (Fray and Grierson, Plant Mol.Biol.22(4),589-602(1993); Fray et al., Plant J., 8, 693-701(1995). As expected, transgenic tobacco plants with reduced amounts of phenylalanine-ammonium lyase show reduced phenylpropanoid amounts. The enzyme phenylalanine-ammonium lyase catalyzes the breakdown of phenylalanine and thus removes it from phenylpropanoid biosynthesis (Bate et al.,Proc. Natl. Acad. Sci USA 91 (16): 7608-7612 (1994); Howles et al., Plant Physiol. 112. 1617-1624(1996).

To date, little has been disclosed about increasing the metabolite flux in order to increase the tocopherol content of plants through overexpression of individual biosynthesis genes. There is merely a description in WO 97/27285 of modification of the tocopherol content by increased expression or by down-regulation of the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD).

It is an object of the present invention to develop a transgenic plant with increased content of tocopherols, vitamin K, chlorophylls and carotenoids.

We have found that this object is achieved by overexpression of a 1-deoxy-D-xylulose-5-phosphate sythase (DOXS) gene in the plants.

In order to increase the metabolite flux from primary metabolism into isoprenoid metabolism, the production of IPP as general starting substrate for all plastidic isoprenoids was increased. For this purpose, the DOXS activity in plants was increased by overexpression of the homologous gene (gene from organism of the same species). This can also be achieved by expressing a heterologous gene (gene from remote organisms). Nucleotide sequences from Arabidopsis thaliana DOXS (Acc. No. U 27099), rice (Acc. No. AF024512) and peppermint (Acc. No. AF019383) are described.

In one example 1 there is enhanced expression of the DOXS gene from Arabidopsis thaliana (SEQ ID No.:1; Mandel et al., Plant J. 9, 649-658(1996); Acc. No. U27099) in transgenic plants. Plastidic localization is ensured by the transit signal sequence present in the gene sequence. A suitable expression cassette is also a DNA sequence which codes for a DOXS gene which hybridizes with SEQ ID No. 1 and which is derived from other organisms such as, for example, E. coli (SEQ ID No.3) or, preferably, from other plants.

The GGPP which is now available in increased quantities is converted further in the direction of tocopherols and carotenoids.

Efficient production of carotenoids is essential for photosynthesis, where they serve together with chlorophylls as "light-collecting complexes" for better utilization of the energy of photons (Heldt, Pflanzenbiochemie. Spektrum Akademischer Verlag Heidelberg Berlin Oxford, 1996). In addition, carotenoids carry out important functions protecting from oxygen free radicals such as singlet oxygen, which they are able to return to

the ground state (Asada, 1994; Demming-Adams and Adams, Trends in Plant Sciences 1; 21-26(1996). A 1-deoxy-D-xylulose-5-phosphate synthase-defective Arabidopsis thaliana mutant showing an "albino phenotype" has been isolated (Mandel et al., 1996). It can be inferred from this that a reduced amount of carotenoids in the plastids has adverse effects on the plant.

We have found that the object is also achieved by overexpression of a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) gene and of a p-hydroxyphenylpyruvate dioxygenase (HPPD) gene in the plants, see Figure 1.

In order to increase the metabolite flux from primary metabolism into isoprenoid metabolism, the production of IPP as general starting substrate for all plastidic isoprenoids was increased. For this purpose, the DOXS activity in transgenic tobacco and oilseed rape plants was increased by overexpression of the DOXS from E. coli. This can be achieved by expression of homologous or other heterologous genes.

The D-1-deoxy-xylulose 5-phosphate which is now available in increased quantities is converted further in the direction of tocopherols and carotenoids.

In addition, the production of homogentisic acid further intensifies the metabolite flux in the direction of phytylquinones and thus tocopherol, see Figure 1. Homogentisic acid is produced from p-hydroxyphenylpyruvate by the enzyme p-hydroxyphenylpyruvate dioxygenase (HPPD). cDNAs coding for this enzyme have been described from various organisms such as, for example, from microorganisms, from plants and from humans.

In Example 11 there was for the first time overexpression of the HPPD gene from Streptomyces avermitilis (Denoya et al., J. Bacteriol. 176(1994), 5312-5319; SEQ ID No. 5) together with the DOXS from E. coli SEQ ID No. 3 in plants and plant plastids.

The increase in the plastidic IPP production leads to enhanced production of all plastidic isoprenoids. The increased provision of homogentisic acid ensures that sufficient substrate is available for the production of tocopherols in the plastids. This homogentisate which is now available in increased quantities can in turn be converted in the transgenic plants with the amount, which is increased due to the overexpression of DOXS, of phytyl diphosphate (PPP). PPP occupies a key position, in this connection, because it serves on the one hand as starting

substrate for chlorophylls and phylloquinones, and on the other hand for tocopherols.

The transgenic plants are produced by transformation of the plants with a construct containing the DOXS and HPPD genes. Tobacco and oilseed rape were employed as model plants for the production of tocopherols, vitamin K, chlorophylls and carotenoids.

The invention also relates to the use of the DNA sequences SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5, which code for a DOXS or HPPD or functional equivalents thereof, for producing a plant with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents. The nucleic acid sequences may in these cases be, for example, DNA or cDNA sequences. Coding sequences suitable for insertion into an expression cassette are, for example, those coding for a DOXS or HPPD and conferring on the host the ability to overproduce tocopherol.

The expression cassettes additionally comprise regulatory nucleic acid sequences which control the expression of the coding sequence in the host cell. In a preferred embodiment, an expression cassette comprises upstream, i.e. at the 5' end of the coding sequence, a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for the DOXS or HPPD gene located in between.

An expression cassette is produced by fusing a suitable promoter to a suitable DOXS or HPPD DNA sequence and preferably a DNA which is inserted between promoter and DOXS or HPPD DNA sequence and codes for a chloroplast-specific transit peptide, and a polyadenylation signal by conventional recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989), and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

It is also possible to use expression cassettes whose DNA sequence codes for a DOXS or HPPD fusion protein, where part of the fusion protein is a transit peptide which controls translocation of the polypeptide. Transit peptides which are specific for chloroplasts and which are eliminated enzymatically

from the DOXS or HPPD part after translocation of the DOXS or HPPD gene into the chloroplasts are preferred. The particularly preferred transit peptide is derived from the plastidic transketolase (TK) or a functional equivalent of this transit peptide (for example the transit peptide of the small subunit of rubisco or ferredoxin-NADP oxidoreductase).

The fused expression cassette coding for a DOXS gene and an HPPD gene is preferably cloned into a vector, for example pBin19, which is suitable for transformation of Agrobacterium tumefaciens.

The invention further relates to the use of an expression cassette comprising DNA sequences SEQ ID No. 1 or SEQ-ID No. 3 and SEQ ID No. 5 or DNA sequences hybridizing with the latter for the transformation of plants or cells, tissues or parts of plants. The preferred aim of the use is to increase the tocopherol, vitamin K, chlorophyll and carotenoid contents of the plant.

It is moreover possible, depending on the choice of the promoter, for expression to take place specifically in the leaves, in the seeds or other parts of the plant. The present invention further relates to such transgenic plants, propagation material thereof and cells, tissues or parts of these plants.

The invention additionally relates to transgenic plants transformed with an expression cassette comprising the sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5 or DNA sequences hybridizing with the latter, and transgenic cells, tissues, parts and propagation material of such plants. Particular preference is given in this connection to transgenic crop plants such as, for example, barley, wheat, rye, corn, oats, soybean, rice, cotton, sugarbeet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and the various tree, nut and vine species.

The invention further relates to:

- Process for transforming a plant, which comprises introducing expression cassettes comprising a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and a DNA sequence SEQ ID No. 5 or DNA sequences hybridizing with the latter into a plant cell, into callus tissue, a whole plant or protoplasts of plants,

- Use of the DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5 or DNA sequences hybridizing with the latter to produce plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents by expression of a DOXS and an HPPD DNA sequence in plants.

The object have also been achieved by overexpression of a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) gene and of a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) gene in the plants, see Figure 1.

In order to increase the metabolite flux from primary metabolism into isoprenoid metabolism, the production of IPP as general starting substrate for all plastidic isoprenoids was increased. For this purpose, the DOXS activity in transgenic tobacco and oilseed rape plants was increased by overexpression of the DOXS from E. coli. This can be achieved by expression of homologous or other heterologous genes.

In order to convert the GGPP which is now available in increased quantities in the direction of tocopherols and carotenoids, in a further step essential to the invention in addition the activity of the enzyme geranylgeranyl-pyrophosphate oxidoreductase is increased by overexpression of a corresponding gene. This measure achieves an increased production of phytyl pyrophosphate through increased conversion of geranylgeranyl pyrophosphate into phytyl pyrophosphate.

This is done, for example, by enhanced expression of the GGPPOR gene from Arabidopsis thaliana (SEQ ID No. 7) in transgenic plants. In order to ensure plastidic localization, a transit signal sequence is put in front of the Arabidopsis GGPPOR. Also suitable as expression cassette is a DNA sequence coding for a GGPPOR gene which hybridizes with SEQ ID No. 7 and which is derived from other organisms or from other plants.

Example 15 describes the cloning of the GGPPOR gene from Arabidopsis thaliana.

Increasing the plastidic 1-deoxy-D-xylulose 5-phosphate and phytyl pyrophosphate production leads to increased production of all plastidic isoprenoids, so that sufficient substrate for the production of tocopherols, chlorophylls, vitamin K and phylloquinones is available in the plastids.

The transgenic plants are produced by transformation of the plants with a construct containing the DOXS and GGPPOR genes. Tobacco and oilseed rape were employed as model plants for the production of tocopherols, vitamin K, chlorophylls and carotenoids.

The invention also relates to the use of the DNA sequences SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 7, which code for a DOXS or GGPPOR or functional equivalents thereof, for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents. The nucleic acid sequences may in these cases be, for example, DNA or cDNA sequences. Coding sequences suitable for insertion into an expression cassette are, for example, those coding for a DOXS or GGPPOR and conferring on the host the ability to overproduce tocopherol.

The expression cassettes additionally comprise regulatory nucleic acid sequences which control the expression of the coding sequence in the host cell. In a preferred embodiment, an expression cassette comprises upstream, i.e. at the 5' end of the coding sequence, a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for the DOXS or GGPPOR gene located in between. Operative linkage means sequential arrangement of promoter, coding sequence, terminator and, where appropriate, further regulatory elements in such a way that each of the regulatory elements can properly carry out its function in the expression of the coding sequence. The sequences which are preferred for the operative linkage, but are not restricted thereto, are targeting sequences to ensure subcellular localization in the apoplast, in the vacuole, in plastids, in the mitochondrion, in the endoplasmic reticulum (ER), in the cell nucleus, in elaioplasts or other compartments and translation enhancers such as the 5' leader sequence from tobacco mosaic virus (Gallie et al., Nucl. Acids Res. 15 (1987), 8693-8711).

For example, the plant expression cassette can be incorporated into the tobacco transformation vector pBinAR-Hyg. Fig. 1 shows the tobacco transformation vectors pBinAR–Hyg with the 35S promoter (A) and pBinAR–Hyg with the seed-specific promoter phaseolin 796 (B):

- HPT: hygromycin phosphotransferase
- OCS: octopine synthase terminator
- PNOS: nopaline synthase promoter

— also drawn in are those restriction cleavage sites which cut the vector only once.

Suitable promoters for the expression cassette are in principle all promoters able to control expression of foreign genes in plants. Preferably used is, in particular, a plant promoter or a promoter derived from a plant virus. The CaMV 35S promoter from cauliflower mosaic virus (Franck et al., Cell 21 (1980), 285-294) is particularly preferred. This promoter contains, as is known, different recognition sequences for transcriptional effectors which, in their totality, lead to permanent and constitutive expression of the inserted gene (Benfey et al., EMBO J. 8 (1989), 2195-2202).

The expression cassette may also contain a chemically inducible promoter by which expression of the exogenous DOXS or GGPPOR gene in the plant can be controlled at a particular time. Promoters of this type, such as the PRP1 promoter (Ward et al., Plant. Mol. Biol. 22 (1993), 361-366), a promoter inducible by salicylic acid (WO 95/19443), a benzenesulfonamide-inducible (EP-A 388186), a tetracycline-inducible (Gatz et al., (1992) Plant J. 2, 397-404), an abscisic acid-inducible (EP-A 335528) and an ethanol- or cyclohexanone-inducible (WO 93/21334) promoter, inter alia, can be used.

Further particularly preferred promoters are those which ensure expression in tissues or parts of plants in which the biosynthesis of tocopherol or its precursors takes place. Particular mention should be made of promoters which ensure leaf-specific expression. Mention should be made of the promoter of cytosolic FBPase from potato or the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8 (1989) 2445 - 245).

An expression cassette is produced by fusing a suitable promoter to a suitable DOXS or GGPPOR DNA sequence and, preferably, to a DNA which is inserted between promoter and DOXS or GGPPOR DNA sequence and which codes for a chloroplast-specific transit peptide, and to a polyadenylation signal, by conventional recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

It is also possible to use expression cassettes whose DNA sequence codes for a DOXS or GGPPOR fusion protein, where part of the fusion protein is a transit peptide which controls translocation of the polypeptide. Transit peptides specific for chloroplasts are particularly preferred, and these are eliminated enzymatically from the DOXS or GGPPOR part after translocation of the DOXS or GGPPOR gene into the chloroplasts. The particularly preferred transit peptide is derived from the plastidic transketolase (TK) or a functional equivalent of this transit peptide (e.g. the transit peptide of the small subunit of rubisco or ferredoxin-NADP oxidoreductase).

The fused expression cassette coding for a DOXS gene or a GGPPOR gene is preferably cloned into a vector, for example pBin19, which is suitable for transforming Agrobacterium tumefaciens.

The invention further relates to the use of an expression cassette comprising DNA sequences SEQ ID No. 1 or SEQ-ID No. 3 and SEQ ID No. 7 or DNA sequences hybridizing with the latter for the transformation of plants or cells, tissues or parts of plants. The preferred aim of the use is to increase the tocopherol, vitamin K, chlorophyll and carotenoid contents of the plant.

It is moreover possible, depending on the choice of the promoter, for expression to take place specifically in the leaves, in the seeds or other parts of the plant. The present invention further relates to such transgenic plants, propagation material thereof and cells, tissues or parts of these plants.

The invention additionally relates to transgenic plants transformed with an expression cassette comprising the sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 7 or DNA sequences hybridizing with the latter, and transgenic cells, tissues, parts and propagation material of such plants. Particular preference is given in this connection to transgenic crop plants such as, for example, barley, wheat, rye, corn, oats, soybean, rice, cotton, sugarbeet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and the various tree, nut and vine species.

The invention further relates to:

- Process for transforming a plant, which comprises introducing expression cassettes comprising a DNA sequence SEQ ID No. 1 or a DNA sequence SEQ ID No. 3 and a SEQ ID No. 7 or DNA

sequences hybridizing with the latter into a plant cell, into callus tissue, a whole plant or protoplasts of plants,

- Use of the DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 7 or DNA sequences hybridizing with the latter to produce plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents by expression of a DOXS and a GGPPOR DNA sequence in plants.

The object have also been achieved by overexpression of a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) gene, a p-hydroxyphenylpyruvate dioxygenase (HPPD) gene and a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) gene in the plants, see Figure 1.

In order to increase the metabolite flux from primary metabolism into isoprenoid metabolism, the production of IPP as general starting substrate for all plastidic isoprenoids was increased. For this purpose, the DOXS activity was increased by overexpression of the DOXS from E. coli in transgenic tobacco and oilseed rape plants. This can also be achieved by expressing homologous or other heterologous DOXS genes - such as, for example, a DNA sequence SEQ ID No. 1.

The D-1-deoxy-xylulose 5-phosphate which is now available in increased quantities is converted further in the direction of geranylgeranyl pyrophosphate.

In order to convert the GGPP which is now available in increased quantities in the direction of tocopherols and carotenoids, in a further step essential to the invention in addition the activity of the enzyme geranylgeranyl pyrophosphate oxidoreductase is increased by overexpression of a corresponding homologous or heterologous gene. This measure achieves an increased production of phytyl pyrophosphate through increased conversion of geranylgeranyl pyrophosphate into phytyl pyrophosphate.

This done, for example, by enhanced expression of the GGPPOR gene from Arabidopsis thaliana (SEQ ID No. 7) in transgenic plants. In order to ensure plastidic localization, a transit signal sequence is put in front of the Arabidopsis GGPPOR. Also suitable as expression cassette is a DNA sequence coding for a GGPPOR gene which hybridizes with SEQ ID No. 7 and which is derived from other organisms or from other plants.

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Example 15 describes the cloning of the GGPPOR gene from Arabidopsis thaliana.

In order to convert the PPP which is now available in increased quantities in the direction of tocopherol and carotenoids, in a further step essential to the invention in addition the activity of the enzyme p-hydroxylphenylpyruvate dioxygenase (HPPD) is increased by overexpression of a corresponding homologous or heterologous gene. This measure achieves increased production of homogentisic acid by increased conversion of hydroxyphenylpyruvate into homogentisic acid.

cDNAs coding for this enzyme have been described from various organisms such as, for example, from microorganisms, from plants and from humans.

Example 10 describes the cloning of the HPPD gene from Streptomyces avermitilis (Denoya et al., J. Bacteriol. 176(1994), 5312-5319; SEQ ID No. 5). In order to ensure a plastidic localization, a transit signal sequence is put in front of the Streptomyces HPPD. Also suitable as expression cassette is a DNA sequence which codes for an HPPD gene which hybridizes with SEQ ID No. 5 and is derived from other organisms or from plants.

The increase in the plastidic D-1-deoxy-xylulose 5-phosphate, the phytyl pyrophosphate and the homogentisic acid production leads to increased production of all plastidic isoprenoids. The increased provision of these precursors ensures that sufficient substrate is available for the production of tocopherols, chlorophylls, vitamin K and phylloquinones in the plastids.

The transgenic plants according to the invention are produced by transforming the plants with a construct containing the DOXS, the HPPD gene and the GGPPOR gene (Example 17). Tobacco and oilseed rape were employed as model plants for producing tocopherols, vitamin K, chlorophylls and carotenoids.

The invention relates to the use of the DNA sequences SEQ ID No. 1 or SEQ ID No. 3, SEQ ID No. 5 and SEQ-ID No. 7, which code for a DOXS, an HPPD and a GGPPOR or functional equivalents thereof to produce a plant with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents. The nucleic acid sequences may in these cases be, for example, DNA or cDNA sequences. Coding sequences suitable for insertion into an expression cassette are, for example, those coding for a DOXS, an HPPD and a GGPPOR and conferring on the host the ability to overproduce tocopherol.

The expression cassettes additionally comprise regulatory nucleic acid sequences which control the expression of the coding sequence in the host cell. In a preferred embodiment, an expression cassette comprises upstream, i.e. at the 5' end of the coding sequence, a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for the DOXS, the HPPD or the GGPPOR gene located in between. Operative linkage means sequential arrangement of promoter, coding sequence, terminator and, where appropriate, further regulatory elements in such a way that each of the regulatory elements can properly carry out its function in the expression of the coding sequence. The sequences which are preferred for the operative linkage, but are not restricted thereto, are targeting sequences to ensure subcellular localization in the apoplast, in the vacuole, in plastids, in the mitochondrion, in the endoplasmic reticulum (ER), in the cell nucleus, in elaioplasts or other compartments and translation enhancers such as the 5' leader sequence from tobacco mosaic virus (Gallie et al., Nucl. Acids Res. 15 (1987), 8693-8711).

For example, the plant expression cassette can be incorporated into the tobacco transformation vector pBinAR-Hyg. Fig. 2 shows the tobacco transformation vectors pBinAR-Hyg with the 35S promoter (A) and pBinAR-Hyg with the seed-specific promoter phaseolin 796 (B):

- HPT: hygromycin phosphotransferase
- OCS: octopine synthase terminator
- PNOS: nopaline synthase promoter
- also drawn in are those restriction cleavage sites which cut the vector only once.

Suitable promoters for the expression cassette are in principle all promoters able to control expression of foreign genes in plants. Preferably used is, in particular, a plant promoter or a promoter derived from a plant virus. The CaMV 35S promoter from cauliflower mosaic virus (Franck et al., Cell 21 (1980), 285-294) is particularly preferred. This promoter contains, as is known, different recognition sequences for transcriptional effectors which, in their totality, lead to permanent and constitutive expression of the inserted gene (Benfey et al., EMBO J. 8 (1989), 2195-2202).

The expression cassette may also contain a chemically inducible promoter by which expression of the exogenous DOXS, HPPD and GGPOR gene in the plant can be controlled at a particular time.

Promoters of this type, such as the PRP1 promoter (Ward et al., Plant. Mol. Biol. 22 (1993), 361-366), a promoter inducible by salicylic acid (WO 95/19443), a benzenesulfonamide-inducible (EP-A 388186), a tetracycline-inducible (Gatz et al., (1992) Plant J. 2, 397-404), an abscisic acid-inducible (EP-A 335528) and an ethanol- or cyclohexanone-inducible (WO 93/21334) promoter, inter alia, can be used.

Further particularly preferred promoters are those which ensure expression in tissues or parts of plants in which the biosynthesis of tocopherol or its precursors takes place. Particular mention should be made of promoters which ensure leaf-specific expression. Mention should be made of the promoter of cytosolic FBPase from potato or the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8 (1989) 2445 - 245).

An expression cassette is produced by fusing a suitable promoter to a suitable DOXS, HPPD and GGPPOR DNA sequence and, preferably, to a DNA which is inserted between promoter and DOXS, HPPD and GGPOR DNA sequence and which codes for a chloroplast-specific transit peptide, and to a polyadenylation signal, by conventional recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

It is also possible to use expression cassettes whose DNA sequence codes for a DOXS, HPPD and GGPOR fusion protein, where part of the fusion protein is a transit peptide which controls translocation of the polypeptide. Transit peptides specific for chloroplasts are particularly preferred, and these are eliminated enzymatically from the DOXS, HPPD and GGPPOR part after translocation of the DOXS, HPPD and GGPOR gene into the chloroplasts. The particularly preferred transit peptide is derived from the plastidic transketolase (TK) or a functional equivalent of this transit peptide (e.g. the transit peptide of the small subunit of rubisco or ferredoxin-NADP oxidoreductase).

The fused expression cassette coding for a DOXS gene, an HPPD gene or a GGPPOR gene is preferably cloned into a vector, for example pBin19, which is suitable for transforming Agrobacterium tumefaciens.

The invention further relates to the use of an expression cassette comprising DNA sequences SEQ ID No. 1 or SEQ-ID No. 3, SEQ ID No. 5 and SEQ ID No. 7 or DNA sequences hybridizing with the latter for the transformation of plants or cells, tissues or parts of plants. The preferred aim of the use is to increase the tocopherol, vitamin K, chlorophyll and carotenoid contents of the plant.

It is moreover possible, depending on the choice of the promoter, for expression to take place specifically in the leaves, in the seeds or other parts of the plant. The present invention further relates to such transgenic plants, propagation material thereof and cells, tissues or parts of these plants.

The invention additionally relates to transgenic plants transformed with an expression cassette comprising the sequence SEQ ID No. 1 or SEQ ID No. 3, SEQ ID No. 5 and SEQ ID No. 7 or DNA sequences hybridizing with the latter, and transgenic cells, tissues, parts and propagation material of such plants. Particular preference is given in this connection to transgenic crop plants such as, for example, barley, wheat, rye, corn, oats, soybean, rice, cotton, sugarbeet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and the various tree, nut and vine species.

The invention further relates to:

- Processes for transforming a plant, which comprises introducing expression cassettes comprising a DNA sequence SEQ ID No. 1 or SEQ ID No. 3, a DNA sequence SEQ ID No. 5 and a DNA sequence SEQ ID No. 7 or DNA sequences hybridizing with the latter into a plant cell, into callus tissue, a whole plant or protoplasts of plants,

- Use of the DNA sequence SEQ ID No. 1 or SEQ ID No. 3, SEQ ID No. 5 and SEQ ID No. 7 or DNA sequences hybridizing with the latter to produce plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents by expression of a DOXS, an HPPD and an GGPPOR DNA sequence in plants.

It was therefore an additional object of the present invention to develop a test system for identifying DOXS inhibitors.

This object has been achieved by expressing a DOXS gene from Arabidopsis or E. coli, or DNA sequences hybridizing therewith, and subsequently testing chemicals for inhibition of the DOXS enzyme activity.

The transgenic plants are produced by transforming the plants with a construct containing the DOXS gene. Arabidopsis and oilseed rape were employed as model plants for the production of tocopherols, vitamin K, chlorophylls and carotenoids.

Cloning of the complete DOXS gene from Arabidopsis takes place by isolating the cDNA (SEQ ID No. 1) specific for the DOXS gene.

The invention relates to the use of the DNA sequence SEQ ID No. 1 or SEQ ID No. 3 which codes for a DOXS or functional equivalent thereof for producing a plant with increased tocopherol, vitamin K, chlorophyll and/or carotenoid content. The nucleic acid sequence can moreover be, for example, a DNA or cDNA sequence. Examples of coding sequences suitable for insertion into an expression cassette are those which code for a DOXS and which confer on the host the ability to overproduce tocopherol.

The expression cassettes additionally comprise regulatory nucleic acid sequences which control the expression of the coding sequence in the host cell. In a preferred embodiment, an expression cassette comprises a promoter upstream, i.e. at the 5' end of the coding sequence, and a polyadenylation signal downstream, i.e. at the 3' end, and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for the DOXS gene located in between. Operative linkage means sequential arrangement of promoter, coding sequence, terminator and, where appropriate, further regulatory elements in such a way that each of the regulatory elements can properly carry out its function in the expression of the coding sequence. The sequences which are preferred for the operative linkage, but are not restricted thereto, are targeting sequences to ensure subcellular localization in the apoplast, in the vacuole, in plastids, in the mitochondrion, in the endoplasmic reticulum (ER), in the cell nucleus, in elaioplasts or other compartments and translation enhancers such as the 5' leader sequence from tobacco mosaic virus (Gallie et al., Nucl. Acids Res. 15 (1987) 8693 - 8711).

For example, the plant expression cassette can be incorporated into the tobacco transformation vector pBinAR-Hyg. Fig. [lacuna] shows the tobacco transformation vectors pBinAR–Hyg with the 35S

promoter (A) and pBinAR-Hyg with the seed-specific promoter phaseolin 796 (B):

- HPT: hygromycin phosphotransferase
- OCS: octopine synthase terminator
- PNOS: nopaline synthase promoter
- also drawn in are those restriction cleavage sites which cut the vector only once.

Suitable promoters for the expression cassette are in principle all promoters able to control expression of foreign genes in plants. Preferably used is, in particular, a plant promoter or a promoter derived from a plant virus. The CaMV 35S promoter from cauliflower mosaic virus (Franck et al., Cell 21 (1980), 285-294) is particularly preferred. This promoter contains, as is known, different recognition sequences for transcriptional effectors which, in their totality, lead to permanent and constitutive expression of the inserted gene (Benfey et al., EMBO J. 8 (1989), 2195-2202).

The expression cassette may also contain a chemically inducible promoter by which expression of the exogenous DOXS gene in the plant can be controlled at a particular time. Promoters of this type, such as the PRP1 promoter (Ward et al., Plant. Mol. Biol. 22 (1993), 361-366), a promoter inducible by salicylic acid (WO 95/19443), a benzenesulfonamide-inducible (EP-A 388186), a tetracycline-inducible (Gatz et al., (1992) Plant J. 2, 397-404), an abscisic acid-inducible (EP-A 335528) and an ethanol- or cyclohexanone-inducible (WO 93/21334) promoter, inter alia, can be used.

Further particularly preferred promoters are those which ensure expression in tissues or parts of plants in which the bio-synthesis of tocopherol or its precursors takes place. Particular mention should be made of promoters which ensure leaf-specific expression. Mention should be made of the promoter of cytosolic FBPase from potato or the ST-LSI promoter from potato (Stockhaus et al., EMBO J. 8 (1989) 2445 - 245).

It has been possible with the aid of a seed-specific promoter to express a foreign protein stably up to a content of 0.67% of the total soluble seed protein in the seeds of transgenic tobacco plants (Fiedler and Conrad, Bio/Technology 10 (1995), 1090-1094). The expression cassette can therefore contain, for example, a seed-specific promoter (preferably the phaseolin promoter (US 5504200), the USP (Baumlein, H. et al. Mol. Gen. Genet. (1991) 225 (3), 459 - 467) or LEB4 promoter (Fiedler and Conrad,

1995)), the LEB4 signal peptide, the gene to be expressed, and an ER retention signal. The construction of a cassette of this type is depicted diagrammatically by way of example in Figure 2.

An expression cassette is produced by fusing a suitable promoter to a suitable DOXS DNA sequence and, preferably, to a DNA which is inserted between promoter and DOXS DNA sequence and which codes for a chloroplast-specific transit peptide, and to a polyadenylation signal, by conventional recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

Particularly preferred sequences are those which ensure targeting in the apoplast, in plastids, in the vacuole, in the mitochondrion, in the endoplasmic reticulum (ER) or, due to absence of appropriate operative sequences, ensure retention in the compartment of production, the cytosol (Kermode, Crit. Rev. Plant Sci. 15, 4 (1996), 285 - 423). Localization in the ER has proved particularly beneficial for the amount of protein accumulated in transgenic plants (Schouten et al., Plant Mol. Biol. 30 (1996), 781 - 792).

It is also possible to use expression cassettes whose DNA sequence codes for a DOXS fusion protein, where part of the fusion protein is a transit peptide which controls translocation of the polypeptide. Transit peptides specific for chloroplasts are particularly preferred, and these are eliminated enzymatically from the DOXS part after translocation of the DOXS gene into the chloroplasts. The particularly preferred transit peptide is derived from the plastidic transketolase (TK) or a functional equivalent of this transit peptide (e.g. the transit peptide of the small subunit of rubisco or ferredoxin-NADP oxidoreductase).

The inserted nucleotide sequence coding for a DOXS can be prepared by synthesis or be obtained naturally or comprise a mixture of synthetic and natural DNA constituents, and may consist of different heterologous DOXS gene sections from different organisms. In general, synthetic nucleotide sequences are produced with codons preferred by plants. These codons preferred by plants can be identified from codons with the highest protein frequency which are expressed in most plant

species of interest. For preparing an expression cassette it is possible to manipulate various DNA fragments in order to obtain a nucleotide sequence which expediently reads in the correct direction and is equipped with a correct reading frame. Adapters or linkers can be attached to the fragments for connecting the DNA fragments to one another.

It is possible and expedient for the promoter and terminator regions to be provided in the direction of transcription with a linker or polylinker which contains one or more restriction sites for inserting this sequence. As a rule, the linker has 1 to 10, usually 1 to 8, preferably 2 to 6, restriction sites. The linker generally has a size of less than 100 bp, frequently less than 60 bp, but at least 5 bp, inside the regulatory regions. The promoter may be both native or homologous and foreign or heterologous to the host plant. The expression cassette comprises in the 5'-3' direction of transcription the promoter, a DNA sequence which codes for a DOXS gene, and a region for termination of transcription. Various termination regions are interchangeable as desired.

It is furthermore possible to employ manipulations which provide appropriate restriction cleavage sites or delete the redundant DNA or restriction cleavage sites. It is possible in relation to insertions, deletions or substitutions, e.g. transitions and transversions, to use *in vitro* mutagenesis, primer repair, restriction or ligation. It is possible with suitable manipulations, e.g. restriction, chewing back or filling in overhangs for blunt ends, to provide complementary ends of the fragments for ligation.

It may be important for success according to the invention inter alia to attach the specific ER retention signal SEKDEL (Schouten, A. et al. Plant Mol. Biol. 30 (1996), 781 - 792), whereby the average level of expression is tripled or quadrupled. It is also possible to employ other retention signals which naturally occur with plant and animal proteins which are localized within the ER for constructing the cassette.

Preferred polyadenylation signals are plant polyadenylation signals, preferably those which essentially correspond to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, especially of gene 3 of the T-DNA (octopine synthase) of the Ti plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984) 835 ff) or functional equivalents.

An expression cassette may comprise, for example, a constitutive promoter (preferably the CaMV 35 S promoter), the LeB4 signal peptide, the gene to be expressed, and the ER retention signal. The ER retention signal preferably used is the amino acid sequence KDEL (lysine, aspartic acid, glutamic acid, leucine).

The fused expression cassette which codes for a DOXS gene is preferably cloned into a vector, for example pBin19, which is suitable for transforming Agrobacterium tumefaciens. Agrobacteria transformed with such a vector can then be used in a known manner for transforming plants, in particular crop plants, e.g. tobacco plants, by, for example, bathing wounded leaves or pieces of leaf in a solution of agrobacteria and then cultivating in suitable media. The transformation of plants by agrobacteria is dislosed inter alia by F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38. Transgenic plants containing a gene, integrated in the expression cassette, for expression of a DOXS gene can be regenerated in a known manner from the transformed cells of the wounded leaves or pieces of leaf.

For transformation of a host plant with a DNA coding for a DOXS, an expression cassette is incorporated as insertion into a recombinant vector whose vector DNA comprises additional functional regulatory signals, for example sequences for replication or integration. Suitable vectors are described inter alia in "Methods in Plant Molecular Biology and Biotechnology" (CRC Press), Chap. 6/7, pp. 71-119 (1993).

It is possible by using the recombination and cloning techniques cited above to clone the expression cassettes into suitable vectors which make their replication possible, for example in *E. coli*. Suitable cloning vectors are, inter alia, pBR332, pUC series, M13mp series and pACYC184. Binary vectors able to replicate both in *E. coli* and in agrobacteria are particularly suitable.

The invention further relates to the use of an expression cassette comprising a DNA sequence SEQ No. 1 or SEQ ID No. 3; SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5; SEQ ID No. 1 or SEQ-ID No. 3 and SEQ-ID No. 7 or a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5 and SEQ ID No. 7, or DNA sequences hybridizing with the latter for transforming plants, or cells, tissues or parts of plants. The aim of the use is preferably to

increase the tocopherol, vitamin K, chlorophyll and carotenoid contents of the plant.

It is moreover possible, depending on the choice of the promoter, for expression to take place specifically in the leaves, in the seeds, or other parts of the plant. The present invention further relates to such transgenic plants, to propagation material thereof and to cells, tissues or parts of the plants.

The expression cassette can in addition be employed for transforming bacteria, cyanobacteria, yeasts, filamentous fungi and algae with the aim of increasing tocopherol, vitamin K, chlorophyll and/or carotenoid production.

The transfer of foreign genes into the genome of a plant is referred to as transformation. In this connection, the described methods for transforming and regenerating plants from plant tissues or plant cells are utilized for transient or stable transformation. Suitable methods are protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic method using the gene gun - called the particle bombardment method, electroporation, incubation of dry embryos in DNA-containing solution, microinjection and gene transfer mediated by *Agrobacterium*. Said processes are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The construct to be expressed is preferably cloned into a vector which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711).

Agrobacteria transformed with an expression cassette can likewise be used in a known manner for transforming plants, in particular crop plants such as cereals, corn, oats, soybean, rice, cotton, sugarbeet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and the various tree, nut and vine species, e.g. by bathing wounded leaves or pieces of leaf in a solution of agrobacteria and subsequently cultivating in suitable media.

Functionally equivalent sequences which code for a DOXS gene are sequences which, despite differing in nucleotide sequence, still have the required functions. Functional equivalents thus comprise naturally occurring variants of the sequences described herein, and artificial artificial nucleotide sequences obtained, for

example, by chemical synthesis and adapted to the codon usage of a plant.

A functional equivalent also means in particular natural or artificial mutations of an originally isolated sequence coding for a DOXS, which still show the required funtion. Mutations comprise substitutions, additions, deletions, transpositions or insertions of one or more nucleotide residues. Thus, the present invention also includes, for example, nucleotide sequences which are obtained by modifying the DOXS nucleotide sequence. The aim of such a modification may be, for example, to localize further the coding sequence present therein or else, for example, to insert further restriction enzyme cleavage sites.

Functional equivalents are also variants whose function is attenuated or enhanced by comparison with the initial gene or gene fragment.

Artificial DNA sequences are also suitable as long as they confer, as described above, the required property, for example of increasing the tocopherol content in the plant by overexpression of the DOXS gene in crop plants. Such artificial DNA sequences can be identified, for example, by back-translation of proteins which have been constructed by molecular modelling and have DOXS activity, or by *in vitro* selection. Particularly suitable coding DNA sequences are those which have been obtained by back-translation of a polypeptide sequence in accordance with the codon usage specific for the host plant. The specific codon usage can easily be established by a skilled worker familiar with plant genetic methods through computer analyses of other, known genes in the plant to be transformed.

Further suitable equivalent nucleic acid sequences which should be mentioned are sequences which code for fusion proteins, in which case a plant DOXS polypeptide or a functionally equivalent part thereof is a constituent of the fusion protein. The second part of the fusion protein can be, for example, another polypeptide with enzymatic activity, or an antigenic polypeptide sequence with whose aid it is possible to detect DOXS expression (e.g. myc tag or his tag). However, this is preferably a regulatory protein sequence, e.g. a signal or transit peptide which guides the DOXS protein to the required site of action.

However, the invention also relates to the expression products generated according to the invention, and to fusion proteins composed of a transit peptide and a polypeptide with DOXS

activity.

Increasing the tocopherol, vitamin K, chlorophyll and/or carotenoid content means for the purpose of the present invention the artificially acquired capability of increased activity in the biosynthesis of these compounds through functional overexpression of the DOXS gene in the plant compared with the plant which has not been genetically modified, for the duration of at least one plant generation.

The site of tocopherol biosynthesis is generally the leaf tissue so that leaf-specific expression of the DOXS gene is sensible. However, it is obvious that tocopherol biosynthesis need not be confined to the leaf tissue, but may also take place tissue-specifically in all other parts of the plant - for example in oilbearing seeds.

Constitutive expression of the exogenous DOXS gene is an additional advantage. However, on the other hand, inducible expression may also appear to be desirable.

The effectiveness of expression of the transgenically expressed DOXS gene can be determined, for example, *in vitro* by shoot meristem propagation. In addition, an alteration in the nature and level of the expression of the DOXS gene and its effect on tocopherol biosynthesis activity can be tested in glasshouse experiments on test plants.

The invention additionally relates to transgenic plants transformed with an expression cassette comprising the sequence SEQ ID No.1 or SEQ ID No. 3; SEQ ID No. 1 or SEQ ID No. 3 and SEQ No. 5; SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 7 or a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5 and SEQ ID No. 7, or DNA sequences hybridizing with the latter, and transgenic cells, tissues, parts and propagation material of such plants. Particularly preferred in this connection are transgenic crop plants such as, for example, barley, wheat, rye, corn, oats, soybean, rice, cotton, sugarbeet, canola, sunflower, flax, hemp, potato, tobacco, tomato, oilseed rape, alfalfa, lettuce and the various tree, nut and vine species.

Plants for the purpose of the invention are mono- and dicotyledonous plants or algae.

In order to be able to find efficient DOXS inhibitors, it is necessary to provide suitable test systems with which inhibitor/enzyme binding studies can be carried out. For this purpose, for

example, the complete cDNA sequence of DOXS from Arabidopsis is cloned into an expression vector (pQE, Qiagen) and overexpressed in *E. coli*.

The DOXS protein expressed using the expression cassette is particularly suitable for finding inhibitors specific for DOXS.

For this purpose, DOXS can be employed, for example, in an enzyme assay in which the activity of DOXS is determined in the presence and absence of the active substance to be tested. Comparison of the two activity determinations allows qualitative and quantitative information to be obtained about the inhibitory behavior of the active substance to be tested. Methods for DOXS activity determination are described (Putra et. al., Tetrahedron Letters 39 (1998), 23-26; Sprenger et al., PNAS 94 (1997), 12857-12862).

The test system according to the invention can be used to examine rapidly and simply a large number of chemical compounds for inhibitory properties. The method allows reproducible selection, from a large number of substances, specifically of those with high activity in order subsequently to carry out with these substances further, more intensive tests familiar to the skilled worker.

It is possible in principle by overexpression of the gene sequence SEQ ID NO: 1 or SEQ ID NO: 3 coding for a DOXS in a plant to achieve increased resistance to DOXS inhibitors. The invention likewise relates to transgenic plants produced in this way.

The invention further relates to:

- A process for transforming a plant, which comprises introducing an expression cassette comprising a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 or a DNA sequence hybridizing with the latter into a plant cell, into callus tissue, a whole plant or protoplasts of plants.

- The use of a plant for producing plant DOXS.

- The use of the expression cassette comprising a DNA sequence SEQ ID No. 1 or SEQ ID NO. 3 or a DNA sequence hybridizing with the latter for producing plants with increased resistance to DOXS inhibitors by enhanced expression of a DNA

sequence SEQ ID No. 1 or SEQ ID NO. 3 or a DNA sequence hybridizing with the latter.

- The use of the DNA sequence SEQ ID No. 1 or SEQ ID NO. 3 or of a DNA sequence hybridizing with the latter for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid content by expression of a DOXS DNA sequence in plants.

- The use of the expression cassette comprising a DNA sequence SEQ ID No. 1 or SEQ ID NO: 3 or a DNA sequence hybridizing with the latter for producing a test system for identifying DOXS inhibitors.

The invention is illustrated by the examples which now follow, but is not confined to these:

General cloning methods

The cloning steps carried out for the purpose of the present invention, such as restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of E. coli cells, cultivation of bacteria, replication of phages and recombinant DNA sequence analysis were carried out as described in Sambrook et al. (1989) Cold Spring Harbor Laboratory Press; ISBN 0-87969-309-6).

The bacterial strains (*E. coli*, XL-I Blue) used below were purchased from Stratagene. The agrobacterium strain used for plant transformation (*Agrobacterium tumefaciens*, C58C1 with the plasmid pGV2260 or pGV3850kann) has been described by Deblaere et al. in (Nucl. Acids Res. 13 (1985) 4777). Alternative possibilities are also to employ the agrobacterium strain LBA4404 (Clontech) or other suitable strains. Vectors which can be used for cloning are pUC19 (Yanish-Perron, Gene 33 (1985), 103-119) pBluescript SK- (Stratagene), pGEM-T (Promega), pZerO (Invitrogen), pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984), 8711-8720) and pBinAR (Höfgen and Willmitzer, Plant Science 66 (1990), 221-230).

Recombinant DNA sequence analysis

Recombinant DNA molecules were sequenced using a Licor laser fluorescence DNA sequencer (marketed by MWG Biotech, Ebersbach)

using the Sanger method (Sanger et al., Proc. Natl. Acad. Sci. USA 74 (1977), 5463-5467).

Example 1

Production of the Arabidopsis thaliana DOXS transformation constructs

The Arabidopsis thaliana DOXS gene was cloned as described in Mandel et al. (1996) as complete cDNA into the vector pBluescript KS- (Stratagene).

To produce overexpression constructs, a 2.3 kb fragment (designated F-23-C) was isolated via the pBluescript KS- HincII (blunt-end) and SacI cleavage sites. This sequence contains the complete DOXS cDNA from the ATG start codon to the EcoRII cleavage site located 80 bp downstream of the stop codon. This fragment was cloned via the SmaI (blunt-end) and SacI cleavage sites into the pBIN19 vector (Figure 3) (Bevan et al., (1980) which contains the 35S promoter of cauliflower mosaic virus (Franck et al., Cell 21(1), 285-294 (1980)) arranged three times in sequence.

To produce antisense constructs, a region of the 3' end of the cDNA (called F-23-C antisense) was cloned into the abovementioned pBIN19-3X35S vector. Part of the 5' region of the DOXS cDNA in pBluescript KS- was digested via HincII and the DOXS-internal BglII cleavage site, and the resulting fragment was removed. (Figure 4). The BglII cleavage site was filled in by the Klenow fill-in reaction (Klenow polymerase; Roche; after reaction according to manufacturer's protocol) so that a blunt end was produced. The ends which were now compatible (BglII blunt end and HinclII were ligated. The 3' region of the DOXS cDNA was then cloned via KpnI and XbaI (both cleavage sites are located in the polylinker of pBluescript KS-5' and 3' of the DOXS cDNA) in antisense orientation into the pBIN19 vector described above in antisense orientation.

Transformations of Arabidopsis thaliana plants with the constructs described above using Agrobacterium tumefaciens took place by the vacuum infiltration method (Bent et al., Science 265 (1994), 1856-1860). Several independent transformands were isolated for each construct. Each letter (see Table 1) denotes an independent transformed line. Plants from the T1 generation obtained therefrom were examined for homo- or heterozygosity. Several plants from each line were crossed in order to carry out a segregation analysis. The number in Table 1 corresponds to the

individual plant selected for further analyses. Both homo- and heterozygous lines were obtained. The segregation analysis of the resulting lines is shown in Table 1 below:

Table 1. Segregation analysis of the transgenic DOXS T2 plants

LINES	SEGREGATION
A9	75%
A19	100%
B11	75%
B4	100%
C2	100%
D3	75%
D17	100%
E9	75%
E14	100%
F9	75%
F14	100%

Example 2

Isolation of genomic DNA of the bacterium Escherichia coli XL1 Blue

A culture of Escherichia coli XL1 Blue was grown in 300 ml of Luria broth medium at 37°C for 12 hours. The genomic DNA of the bacterium was isolated from this culture by first pelleting it at 5 000 revolutions in a Sorvall RC50 fuge. The pellet was then resuspended in 1/30 of the volume of the original culture of lysis buffer (25 mM EDTA, 0.5% SDS; 50 mM Tris HCl, pH 8.0). An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added and incubated at 70 degrees for 10 minutes. The aqueous phase was then separated from the phenolic in a Heraeus floor centrifuge at 3 500 rev for 15 minutes. The aqueous supernatant was mixed with 2.5 volumes of ethanol and 1/10 volume of 8 M lithium chloride, and the nucleic acids were precipitated at room temperature for 10 minutes. The pellet was then taken up in 400 µl of TE/RNAse and incubated at 37 degrees for 10 minutes. The solution was again shaken with one volume of phenol/ chloroform/isoamyl alcohol (25:24:1), and the supernatant was precipitated with 2.5 volumes of ethanol and 1/10 volume of 8 M lithium chloride. The pellet was then washed with 80% ethanol and taken up in 400 µl of TE/RNAse.

Example 3

Isolation of the DOXS from E. coli

Oligonucleotides for a PCR were derived from the DOXS DNA sequence (Acc. Number AF035440), and a BamHI restriction cleavage site was attached to them at the 5' end, and an XbaI or another BamHI restriction cleavage site was attached to them at the 3' end. The oligonucleotide at the 5' end comprises the sequence 5'-ATGGATCC*ATGAGTTTT-GATATTGCCAAATAC*-3' (nucleotides 1-24 of the DNA sequence; in italics) starting with the ATG start codon of the gene, and the oligonucleotide at the 3' end comprises the sequence 5'-ATTCTAG*ATTATGCCAGCCAGGCCTTG*-3' or 5'-ATGGATCC*TTATGCCAGCCAGGCCTTG*-3' (nucleotides 1845-1863 of the reverse complementary DNA sequence; in italics) starting with the stop codon of the gene. The PCR reaction with the two BamHI-containing oligonucleotides was carried out with Pfu polymerase (Stratagene GmbH, Heidelberg) in accordance with the manufacturer's information. 500 ng of the genomic DNA from E. coli were employed as template. The PCR program was as follows:

5 cycles: 4 sec 94°C, 30 sec 52°C, 2 min 72°C;
5 cycles: 4 sec 94°C, 30 sec 48°C, 2 min 72°C;
25 cycles: 4 sec 94°C, 30 sec 44°C, 2 min 72°C

The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script (Stratagene GmbH, Heidelberg). The correctness of the sequence was established by sequencing. The fragment was BamHI isolated from the PCR-Script vector and ligated into a correspondingly cut Bin19 vector which additionally contains the transit peptide of potato transketalase downstream of the CaMV 35S as promoter. The transit peptide ensures plastidic localization. The constructs are depicted in Figure 5 and 6, and the fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of cauliflower mosaic virus (nucleotides 6909 to 7437 of cauliflower mosaic virus). Fragment B (259 bp) comprises the transit peptide of transketolase. Fragment E comprises the DOXS gene. Fragment D (192 bp) comprises the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) to terminate transcription.

The PCR reaction with the 5'-BamHI and 3'-XbaI-containing oligonucleotides was carried out with Taq polymerase (Takara, Sosei Co., Ltd.) in accordance with the manufacturer's information. 500 ng of the genomic DNA from E. coli were employed as template. The PCR program was as follows:

5 cycles: 4 sec 94°C, 4 sec 50°C, 2 min 30°C
5 cycles: 4 sec 94°C, 30 sec 46°C, 2 min 68°C
25 cycles: 4 sec 94°C, 30 sec 42°C, 2 min 68°C

The fragment was purified using a Gene-Clean kit and ligated into the vector pGemT (Promega GmbH, Mannheim). It was cloned as BamHI/XbaI fragment into a correspondingly cut pBin19AR vector downstream of the CaMV 35S promoter. The sequence was checked by sequencing (SEQ-ID No. 3). This revealed two non-conservative base exchanges which, compared with the published sequence, lead to a change in amino acid 152 (asparagine) to valine and amino acid 330 (cysteine) to tryptophan.

Example 4

Detection of increased amounts of DOXS RNA in transgenic plants

Total RNA from 15-day old seedlings of various transgenic lines possessing the DOXS overexpression construct was extracted by the method of Logeman et al., Anal. Biochem. 163, 16-20 (1987), fractionated in a 1.2% agarose gel, transferred to filters and hybridized with a 2.1 kb long DOXS fragment as probe (Figure 7).

Example 5

Detection of increased amounts of DOXS protein in transgenic plants

Total protein (Figure 8) from 15-day old seedlings of various independent transgenic plants possessing the DOXS overexpression construct was isolated and detected in a Western analysis using a polyclonal anti-DOXS antibody (IgG) (Figure 9).

Example 6

Measurement of the carotenoid and chlorophyll contents

The total amounts of carotenoids and chlorophylls were determined as described by Lichtenthaler and Wellburn (1983) using 100% acetone extracts. The results of the multiple measurements of the

transgenic lines possessing the DOXS overexpression construct are shown in Table 2 below.

Table 2: Total carotenoid and chlorophyll contents of transgenic DOXS lines

LINE	% TOTAL CHLOROPHYLLS	% TOTAL CAROTENOIDS
cla1 mutant	5	5
Wild type	100	100
B-4	86	89
B-11	84	90
C-2	98	107
D-3	128	135
D-17	136	149
E-14	121	139
F-7	80	90
F-14	85	107

Example 7

Transformation of oilseed rape

The production of transgenic oilseed rape plants is based on a protocol of Bade, JB and Damm, B (in Gene, Transfer to Plants, Potrykus, I. and Spangenberg, G., eds, Springer Lab Manual, Springer Verlag, 1995, 30-38), in which the composition of the media used are also stated. The transformations took place with the agrobacterium strain LBA4404 (Clontech). The binary vectors used were the pBIN19 constructs with the complete DOXS cDNA already described above. The NOS terminator sequence in these pBIN vectors was replaced by the OCR terminator sequence.

Brassica napus seeds were surface-sterilized with 70% (v/v) ethanol, washed in H_2O at 55°C for 10 min, incubated in 1% strength hypochlorite solution (25% v/v Teepol, 0.1% v/v Twenn 20) for 20 min and washed six times with sterile H_2O for 20 min each time. The seeds were dried on filter paper for three days and 10-15 seeds were induced to germinate in a glass flask with 15 ml of germination medium. The roots and apices were removed from several seedlings (about 10 cm in size), and the remaining hypocotyls were cut into pieces about 6 mm long. The approx. 600 explants obtained in this way are washed with 50 ml of basal medium for 30 min and transferred into a 300 ml flask. After addition of 100 ml of callus induction medium, the cultures were incubated at 100 rpm for 24 h.

An overnight culture of the agrobacterium strain was set up in LB with kanamycin (20 mg/l) at 29°C, and 2 ml of this were incubated in 50 ml of LB without kanamycin at 29°C for 4 h until the OD_{600} was 0.4-0.5. After pelleting of the culture at 2 000 rpm for 25 min, the cell pellet was resuspended in 25 ml of basal medium. The concentration of the bacteria in the solution was adjusted to an OD600 of 0.3 by adding further basal medium.

The callus induction medium was removed from the oilseed rape explants using sterile pipettes, 50 ml of agrobacterium solution were added and, after cautious mixing, incubated for 20 min. The agrobacteria suspension was removed, the oilseed rape explants were washed with 50 ml of callus induction medium for 1 min and then 100 ml of callus induction medium were added. The cocultivation was carried out on a rotary shaker at 100 rpm for 24 h. The cocultivation was stopped by removing the callus induction medium, and the explants were washed twice for 1 min each time with 25 ml and twice for 60 min with 100 ml each time of washing medium at 100 rpm. The washing medium with the explants was transferred into 15 cm Petri dishes, and the medium was removed using sterile pipettes. For regeneration, in each case 20-30 explants were transferred into 90 mm Petri dishes which contained 25 ml of shoot-induction medium with kanamycin. The Petri dishes were sealed with 2 layers of Leukopor and incubated at 25°C and with 2000 lux in 16/8 H photoperiods. The calli which developed was transferred every 12 days to fresh Petri dishes with shoot-induction medium. All further steps for regenerating whole plants ~~were~~ carried out as described by Bade, J.B. and Damm, B. (in Gene Transfer to Plants, Potrykus, I. and Spangenberg, G.,eds, Springer Lab Manual, Springer Verlag, 1995, 30-38).

Example 8

Increasing tocopherol biosynthesis in oilseed rape

The DOXS cDNA (SEQ-ID No. 1) was provided with a CaMV 35S promoter and over-expressed in oilseed rape using the 35S promoter. In parallel with this, the seed-specific promoter of the phaseolin gene was used in order specifically to increase the tocopherol content in the rapeseed. Oilseed rape plants transformed with the corresponding constructs were grown in a glasshouse. The α-tocopherol content of the whole plant and of the seeds of the plant was then determined. In all cases, the α-tocopherol concentration was increased by comparison with the untransformed plant.

Example 9

Detection of the expression of DOXS from E. coli in transgenic tobacco plants

Leaf disks with a diameter of 0.9 cm were taken from completely unfolded leaves of plants containing the construct pBinAR HPPD-DOXS, and were frozen in liquid nitrogen. The leaf material was homogenized in an HEPES-KOH buffer containing proteinase inhibitors, and the protein concentration was determined from the extract using the Bio-Rad protein assay in accordance with the manufacturer's information. 45 µg of protein from each extract were mixed with one volume of loading buffer (Laemmli, 1970) and incubated at 95°C for 5 min. The proteins were then fractionated on a 12.5 percent SDS-PAGE gel. The proteins were then transferred by means of semi-dry electroblots to Porablot membrane (Machery und Nagel). Detection of the DOXS protein took place using a rabbit antibody against E. coli DOXS. The color reaction is based on the binding of a secondary antibody and of an alkaline phosphatase which converts NBT/BCIP into a dye. Secondary antibody and alkaline phosphatase were obtained from Pierce, and the procedure was in accordance with the manufacturer's information.

Figure 10 shows the detection of the DOXS protein in leaves of transgenic plants. 1: marker; 2: plant 10; 3:62; 4: 63; 5: 69; 7:71; 8:112; 9:113; 10:116; 11:WT1; 12:WT2; 13:100 ng of recombinant protein; 14:50 ng of recombinant protein; 15: 10 ng of recombinant protein.

Example 10

Cloning of an HPPD gene from Streptomyces avermitilis U11864

Isolation of genomic DNA of the bacterium Streptomyces avermitilis U11864:

A culture of Streptomyces avermitilis U11864 was grown in 300 ml of YEME medium (5 g of malt extract, 2 g of yeast extract, 2 g of glucose) at 28°C for 96 h. The genomic DNA of the bacterium was isolated from this culture by pelleting it initially at 5000 rev in a Sorvall RC5C fuge. The pellet was then resuspended in 1/30 of the volume of lysis buffer (25 mM EDTA, 0.5% SDS, 50 mM Tris-HCl, pH 8.0). An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added and incubated at 70°C for 10 minutes. The aqueous phase was then separated from the phenolic in a Heraeus floor centrifuge at 3 500 rev for 15 minutes. The aqueous

supernatant was mixed with 2.5 volumes of ethanol in 1/10 volume of 8 M lithium chloride, and the nucleic acids were precipitated at room temperature for 10 minutes. The pellet was then taken up in 400 µl of TE/RNAse and incubated at 37 degrees for 10 minutes. The solution was again shaken with one volume of phenol/chloroform/isoamyl alcohol (25:24:1), and the supernatant was precipitated with 2.5 volumes of ethanol and 1/10 volume of 8 M lithium chloride. The pellet was then washed with 80% ethanol and taken up in 400 µl of TE/RNAse.

Oligonucleotides were derived for a PCR from the DNA sequence of the HPPD from Streptomyces avermitilis (Denoya et al., 1944; Acc. Number U11864), and a BamHI restriction cleavage site was attached to the 5' end of them and an XbaI restriction cleavage site was attached at the 3' end of them. The oligonucleotide at the 5' end comprises the sequence 5'-GG*ATCCAGCGGACAAGCCAAC*-3' (37 to 55 bases distant from the ATG in the 5' direction; in italics), and the oligonucleotide at the 3' end comprises the sequence 5'-TCTAG*ATTATGCCAGCCAGGCCTTG*-3' (nucleotides 1845-1863 of the reverse complementary DNA sequence; in italics).

The PCR reaction was carried out with Pfu polymerase (Stratagene GmbH, Heidelberg) in accordance with the manufacturer's information. 400 ng of the genomic DNA was employed as pattern. The PCR program was as follows:

5 cycles: 4 sec 94°C, 30 sec 54°C, 2 min 72°C
5 cycles: 4 sec 94°C, 30 sec 52°C, 2 min 72°C
25 cycles: 4 sec 94°C, 30 sec 50°C, 2 min 72°C

The fragment was purified by means of a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script (Stratagene GmbH, Heidelberg). The correctness of the sequence was checked by sequencing. This revealed that the isolated gene codes for an additional amino acid. It contains the three bases TAC (coding for tyrosine) in front of nucleotide N429 in the quoted sequence (Denoya et al., 1994).

The fragment was isolated by a BamHI and XbaI digestion from the vector and ligated into a correspondingly cut Bin19AR vector downstream of the CaMV 35S promoter for expression of the gene in the cytosol. The gene was isolated as BamHI fragment from the same PCR-Script vector and was ligated into a correspondingly cut pBin19 vector which additionally comprises the transit peptide of the potato plastidic transketolase downstream of the CaMV 35S promoter. The transit peptide ensures the plastidic localization.

The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script (Stratagene GmbH, Heidelberg). The correctness of the sequence was checked by sequencing. It was cut as BamHI fragment out of the vector PCR-Script and ligated into a correspondingly cut pBinAR vector which additionally contains the transit peptide of transketolase for introducing the gene product into the plastids. The result was the plasmid pBinAR-TP-HPPD (Figure 12).

For the cloning, the 35S promoter, the transketolase transit peptide, the HPPD gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al. 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-HPPD by PCR. A HindIII cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the 5' region of the promoter (in italics) is 5'-ATAAGCTT*CATGGAGTCAAA-GATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the termination sequence (in italics) is 5'-ATAAGCTT*GGACAATCAGTAAATTGAACGGAG*-3'. The resulting fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred as HindIII fragment from this PCR-Script vector into the correspondingly cut vector pBin19 (Bevan, 1984, Nucleic Acids Res. 12, 8711-8721).

The 35S promoter, the transketolase transit peptide, the DOXS gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated by PCR from the plasmid pBinAR-TP-DOXS. An EcoRI cleavage site was attached to each of the oligonucleotides for the promoter and terminator sequence. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'-ATGAATTC*CATGGAGTCAAAGATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'-ATGAATTC*GGACAATCAGTAAATTGAA-CGGAG*-3'. The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script (Stratagene GmbH, Heidelberg). The correctness of the sequence was checked by sequencing (SEQ ID No. 3). It was transferred as EcoRI fragment from the PCR-Script vector into the correspondingly cut vector pBin19 (Bevan, 1984).

It was transferred as XbaI fragment from the PCR-Script vector into the correspondingly cut vector which, as described above, already contains the HPPD sequence. The result was the construct pBinAR-HPPD-DOXS (Figure 13), whose fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of the cauliflower mosaic virus (nucleotides 6909 to 7437). Fragment B comprises the transit peptide of plastidic transketolase. Fragment C comprises the HPPD gene. Fragment D comprises the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Fragment E comprises the DOXS gene.

Example 12

Production of transgenic tobacco plants
(Nicotiana tabacum L. cv. Samsun NN)

Transgenic tobacco plants having an altered prenyl lipid content were produced by transforming tobacco leaf disks with DOXS and HPPD sequences. To transform tobacco plants, 10 ml of an Agrobacterium tumefaciens overnight culture which had grown under selection were centrifuged, the supernatant was discarded and the bacteria were resuspended in the same volume of antibiotic-free medium. Leaf disks from sterile plants (diameter about 1 cm) were bathed in this bacterial suspension in a sterile Petri dish. The leaf disks were then placed on MS medium (Murashige and Skoog, Physiol. Plant (1962) 15, 473) with 2% sucrose and 0.8% Bacto agar in Petri dishes. After incubation in the dark at 25°C for 2 days, they were transferred to MS medium with 100 mg/l kanamycin, 500 mg/l Claforan, 1 mg/l benzylaminopurine (BAP), 0.2 mg/l naphthylacetic acid (NAA), 1.6% glucose and 0.8% Bacto agar, and the cultivation was continued (16 hours of light/ 8 hours of dark). Growing shoots were transferred to hormone-free MS medium with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar.

Example 13

Production of transgenic oilseed rape plants (Brassica napus)

The production of transgenic oilseed rape plants having an altered prenyl lipid content was based on a protocol by Bade, J.B. and Damm, B. (in Gene Transfer to Plants, Potrykus, I. and Spangenberg, G., eds, Springer Lab Manual, Springer Verlag,

1995, 30-38), in which the compositions of the media and buffers used are also indicated.

The transformations took place with the Agrobacterium tumefaciens strain LBA4404 (Clontech GmbH, Heidelberg). The binary vectors used were the binary constructs already described above with the total cDNAs of DOXS and HPPD. In all the binary vectors used here, the NOS terminator sequence was replaced by the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Brassica napus seeds were surface-sterilized with 70% (v/v) ethanol, washed in H_2O at 55°C for 10 min, incubated in 1% strength hypochlorite solution (25% v/v Teepol, 0.1% v/v Tween 20) for 20 min and washed six times with sterile H_2O for 20 min each time. The seeds were dried on filter paper for three days and 10-15 seeds were induced to germinate in a glass flask with 15 ml of germination medium. The roots and apices were removed from several seedlings (about 10 cm in size), and the remaining hypocotyls were cut into pieces about 6 mm long. The approx. 600 explants obtained in this way are washed with 50 ml of basal medium for 30 min and transferred into a 300 ml flask. After addition of 100 ml of callus induction medium, the cultures were incubated at 100 rpm for 24 h.

An overnight culture of the agrobacterium strain was set up in Luria Broth medium with kanamycin (20 mg/l) at 29°C, and 2 ml of this were incubated in 50 ml of Luria Broth medium without kanamycin at 29°C for 4 h until the OD_{600} was 0.4-0.5. After pelleting of the culture at 2 000 rpm for 25 min, the cell pellet was resuspended in 25 ml of basal medium. The concentration of the bacteria in the solution was adjusted to an OD_{600} of 0.3 by adding further basal medium.

The callus induction medium was removed from the oilseed rape explants using sterile pipettes, 50 ml of agrobacterium solution were added and, after cautious mixing, incubated for 20 min. The agrobacteria suspension was removed, the oilseed rape explants were washed with 50 ml of callus induction medium for 1 min and then 100 ml of callus induction medium were added. The cocultivation was carried out on a rotary shaker at 100 rpm for 24 h. The cocultivation was stopped by removing the callus induction medium, and the explants were washed twice for 1 min each time with 25 ml and twice for 60 min with 100 ml each time of washing medium at 100 rpm. The washing medium with the explants was transferred into 15 cm Petri dishes, and the medium was removed using sterile pipettes.

For regeneration, in each case 20-30 explants were transferred into 90 mm Petri dishes which contained 25 ml of shoot-induction medium with kanamycin. The Petri dishes were sealed with 2 layers of Leukopor and incubated at 25°C and with 2 000 lux in photoperiods of 16 hours of light/8 hours of dark. The calli which developed were transferred every 12 days to fresh Petri dishes with shoot-induction medium. All further steps for regenerating whole plants ~~were~~ carried out as described by Bade, J.B. and Damm, B. (in Gene Transfer to Plants, Potrykus, I. and Spangenberg, G.,eds, Springer Lab Manual, Springer Verlag, 1995, 30-38).

Example 14

Increasing tocopherol biosynthesis in oilseed rape

The cDNA of DOXS (SEQ-ID No. 3) and of HPPD (SEQ-ID No. 5) was provided with a CaMV35S promoter and overexpressed in oilseed rape using the 35S promoter. In parallel with this, the seed-specific promoter of the phaseolin gene was used in order specifically to increase the tocopherol content in the rape seed. Oilseed rape plants transformed with the corresponding constructs were grown in a glasshouse. The α-tocopherol content of the whole plant and of the seeds of the plant was then determined. In all cases, the α-tocopherol concentration was increased by comparison with the untransformed plant.

Example 15

Cloning of a GGPPOR gene from Arabidopsis thaliana

Isolation of total RNA from completely unfolded leaves of Arabidopsis thaliana:

Completely unfolded leaves of Arabidopsis thaliana were harvested and frozen in liquid nitrogen. The material was then powdered in a mortar and taken up in Z6 buffer (8 M guanidium hydrochloride, 20 mM MES, 20 mM EDTA pH 7.0). The suspension was transferred into reaction vessels and shaken with one volume of phenol/ chloroform/isoamyl alcohol 25:24:1). After centrifugation at 15 000 rpm for 10 minutes, the supernatant was transferred into a new reaction vessel, and the RNA was precipitated with 1/20 volumes of 1N acetic acid and 0.7 volume of ethanol (absolute). After renewed centrifugation, the pellet was first washed with 3M sodium acetate solution and, after a further centrifugation, in 70% ethanol. The pellet was then dissolved in

confers on plants resistance to the antibiotic hygromycin and is thus suitable for superinfection of plants with kanamycin resistance. Since the plastid transit peptide of GGPPOR was also cloned, the protein ought to be transported into the plastids in transgenic plants. The construct is depicted in Figure 14. The fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of cauliflower mosaic virus (nucleotides 6909 to 7437 of cauliflower mosaic virus). Fragment D comprises the polyadenylation signal of gene 3 of the T DNA of Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Fragment F comprises the gene of GGPPOR including the intrinsic plastid transit sequence.

Example 16

Production of constructs for transformation of plants with DOXS and GGPPOR sequences

To produce plants which are transgenic for DOXS and GGPPOR, a binary vector comprising both gene sequences was manufactured (Figure 15). The GGPPOR gene with the intrinsic plastidic localization sequence was cloned (as described in Example 15) as BamHI/SalI fragment into the correspondingly cut vector pBinAR-Hyg. The DOXS gene was cloned as BamHI fragment as described in Example 3. The vector pBinAR-Hyg contains the 35S promoter of cauliflower mosaic virus and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. This plasmid confers on plants resistance to the antibody hygromycin and is thus suitable for superinfection of plants with kanamycin resistance.

The 35S promoter, the transketolase transit peptide, the DOXS gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-DOXS by PCR. An EcoRI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator sequence. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'-ATGAATTC*CATGGAGTCAAAGATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'-ATGAATTC*GGACAATCAGTAAATTGAACGGAG*-3'. The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by

sequencing. It was transferred from the PCR-Script vector as EcoRI fragment into the correspondingly cut vector pBin19 (Bevan, Nucleic Acids Res. 12 (1984), 8711-8721).

The 35S promoter, the GGPPOR gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinARHyg-GGPPOR by PCR. An XbaI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'-ATTCTAGA*CATGGAGTCAAA-GATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'-ATTCTAGA*GGACAA-TCAGTAAATTGAACGGAG*-3'. The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information onto the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as XbaI fragment into the correspondingly cut vector which already contained, as described above, the DOXS sequence. The result was the construct pBinAR-DOXS-GGPPOR (Figure 15), whose fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of cauliflower mosaic virus (nucleotides 6909 to 7437 of cauliflower mosaic virus). Fragment B comprises the transit peptide of the plastidic transketolase. Fragment E comprises the DOXS gene. Fragment D comprises the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Fragment F comprises the GGPPOR gene including the intrinsic plastid transit sequence.

Example 17

Production of constructs for transformation of plants with DOXS, GGPPOR and HPPD DNA sequences

To produce plants which are transgenic for DOXS, GGPPOR and HPPD, a binary vector containing all three gene sequences was manufactured (Figure 16). The GGPPOR gene was provided with the intrinsic plastidic localization sequence (as described in Example 15). The pBinAR-Hyg vector used confers on plants resistance to the antibiotic hygromycin and is thus suitable for superinfection of plants with kanamycin resistance.

To clone HPPD into vectors which additionally contain another cDNA, oligonucleotides were derived for a PCR, and a BamHI restriction cleavage site was attached to them at the 5' end and 3' end. The oligonucleotide at the 5' end comprises the sequence 5'-GG*ATCCTCCAGCGGACAAGCCAAC*-3' (nucleotides 37 to 55 distant from ATG in the 5' direction; in italics), and the oligonucleotide at the 3' end comprises the sequence 5'-ATGGATCC*CGCGCCGCCTACAGGTTG*-3' (ending with base pair 1140 of the coding sequence, starting 8 base pairs 3' of the TAG stop codon; in italics). The PCR reaction was carried out with Tli polymerase from Promega GmbH, Mannheim in accordance with the manufacturer's information. 10 ng of the plasmid pBinAR-HPPD were employed as template. The PCR program was as follows:

5 cycles: 94°C 4 sec, 68°C 30 sec, 72°C 2 min
5 cycles: 94°C 4 sec, 64°C 30 sec, 72°C 2 min
25 cycles: 94°C 4 sec, 60°C 30 sec, 72°C 2 min

The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was cut out of the vector PCR-Script as BamHI fragment and ligated into a correspondingly cut pBinAR vector which additionally contains the transit peptide of transketolase for introducing the gene product into plastids. The result was the plasmid pBinAR-TP-p-HPPD.

For the cloning, the 35S promoter, the transketolase transit peptide, the p-HPPD gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al. 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-p-HPPD by PCR. A HindIII cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the 5' region of the promoter (in italics) is 5'-ATAAGCTT*CATGGAGTCAAA-GATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the termination sequence (in italics) is 5'-ATAAGCTT*GGAC-AATCAGTAAATTGAACGGAG*-3'. The resulting fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred as HindIII fragment from this PCR-Script vector into the correspondingly cut vector pBin19 (Bevan, 1984, Nucleic Acids Res. 12, 8711-8721).

The 35S promoter, the transketolase transit peptide, the DOXS gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-DOXS by PCR. An EcoRI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator sequence. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'-ATGAATTC*CATGGAGTCAAAGATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'-ATGAATTC*GGACAATCAGTAAATTGAACGGAG*-3'. The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as EcoRI fragment into the correspondingly cut vector which already contained the HPPD sequence as described above.

The 35S promoter, the GGPPOR gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinARHyg-GGPPOR by PCR. An XbaI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'-ATTCTAGA*CATGGAGTCAAA-GATTCAAATAGA*-3', and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'-ATTCTAGA*GGACAA-TCAGTAAATTGAACGGAG*-3'. The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as XbaI fragment into the correspondingly cut vector which already contained the HPPD and DOXS sequences as described above. The result was the construct pBinAR-DOXS-GGPPOR-HPPD (Figure 16), whose fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of cauliflower mosaic virus (nucleotides 6909 to 7437 of cauliflower mosaic virus). Fragment B comprises the transit peptide of the plastidic transketolase. Fragment C comprises the HPPD gene. Fragment D comprises the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Fragment E comprises the DOXS gene. Fragment F

comprises the GGPPOR gene including the intrinsic plastid transit sequence.

Example 18

Increasing tocopherol biosynthesis in oilseed rape

The cDNA of DOXS (SEQ ID No. 3) and of GGPOR (SEQ ID No. 7) was provided with a CaMV35S promoter and overexpressed in rape using the 35S promoter. In parallel with this, the seed-specific promoter of the phaseolin gene was used in order specifically to increase the tocopherol content in the rapeseed. Oilseed rape plants transformed with the corresponding constructs were grown in a glasshouse. The α-tocopherol content of the whole plant and of the seeds of the plant was then determined. In all cases, the α-tocopherol concentration was increased by comparison with the untransformed plant.

Example 19

Increasing the tocopherol biosynthesis in oilseed rape

The cDNA of DOXS (SEQ ID No. 3), of HPPD (SEQ ID No. 5) and of GGPPOR (SEQ-ID No. 7) was provided with a CaMV35S promoter and overexpressed in rape using the 35S promoter. In parallel with this, the seed-specific promoter of the phaseolin gene was used in order specifically to increase the tocopherol content in the rapeseed. Oilseed rape plants transformed with the corresponding constructs were grown in a glasshouse. The α-tocopherol content of the whole plant and of the seeds of the plant was then determined. In all cases, the α-tocopherol concentration was increased by comparison with the untransformed plant.

We claim:

1. The use of DNA sequences coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents.

2. The use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 or of a DNA sequence which hybridizes with the latter and codes for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) for producing plants with increased content of tocopherols, vitamin K, chlorophylls and/or carotenoids.

3. The use of DNA sequences coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and coding for a p-hydroxyphenylpyruvate dioxygenase (HPPD) for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents.

4. The use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and of a DNA sequence SEQ ID No. 5 or of a DNA sequence which hybridizes with the latter and codes for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and a p-hydroxyphenylpyruvate dioxygenase for producing plants with increased content of tocopherols, vitamin K, chlorophylls and/or carotenoids.

5. The use of DNA sequences coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and coding for a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents.

6. The use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and of a DNA sequence SEQ ID No. 7 or of a DNA sequence which hybridizes with the latter and codes for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents.

7. The use of DNA sequences coding for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS) and coding for a hydroxyphenylpyruvate dioxygenase (HPPD) and coding for a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) for

producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents.

8. The use of a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and of a DNA sequence SEQ ID No. 5 and of a DNA sequence SEQ ID No. 7 or of a DNA sequence which hybridizes with the latter and codes for a 1-deoxy-D-xylulose-5-phosphate synthase (DOXS), a hydroxyphenylpyruvate dioxygenase (HPPD) and a geranylgeranyl-pyrophosphate oxidoreductase (GGPPOR) for producing plants with increased content of tocopherols, vitamin K, chlorophylls and/or carotenoids.

9. A process for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents, which comprises expressing a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 or a DNA sequence which hybridizes with the latter in plants.

10. A process for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents, which comprises expressing a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and a DNA sequence SEQ ID No. 5 or DNA sequences which hybridize with the latter in plants.

11. A process for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents, which comprises expressing a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 and a DNA sequence SEQ ID No. 7 or DNA sequences which hybridize with the latter in plants.

12. A process for producing plants with increased tocopherol, vitamin K, chlorophyll and/or carotenoid contents, which comprises expressing DNA sequences SEQ ID No. 1 or SEQ ID No. 3, SEQ ID No. 5 and SEQ ID No. 7 or DNA sequences which hybridize with the latter in plants.

13. A process for transforming a plant, which comprises introducing an expression cassette comprising a promoter and a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 into a plant cell, into callus tissue, a whole plant or protoplasts of plant cells.

14. A process for transforming a plant, which comprises introducing an expression cassette comprising a promoter and DNA sequences SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 5

into a plant cell, into callus tissue, a whole plant or protoplasts of plant cells.

15. A process for transforming a plant, which comprises introducing an expression cassette comprising a promoter and DNA sequences SEQ ID No. 1 or SEQ ID No. 3 and SEQ ID No. 7 into a plant cell, into callus tissue, a whole plant or protoplasts of plant cells.

16. A process for transforming a plant, which comprises introducing an expression cassette comprising a promoter and DNA sequences SEQ ID No. 1 or SEQ ID No. 3, SEQ ID No. 5 and SEQ ID No. 7 into a plant cell, into callus tissue, a whole plant or protoplasts of plant cells.

17. A process for transforming plants as claimed in claim 13-16, wherein the transformation takes place with the aid of the strain Agrobacterium tumefaciens, of electroporation or of the particle bombardment method.

18. A plant transformed with an expression cassette as set forth in claim 13-16.

19. A plant as claimed in claim 18 selected from the group of soybean, canola, barley, oats, wheat, oilseed rape, corn or sunflower.

20. The use of SEQ ID No. 1 or SEQ-ID No. 3 for producing a test system for identifying DOXS inhibitors

21. A test system based on the expression of an expression cassette as set forth in claim 13 for identifying DOXS inhibitors.

22. The use of a plant comprising a DNA sequence SEQ ID No. 1 or SEQ ID No. 3 or a DNA sequence which hybridize with the latter for producing plant and bacterial DOXS.

SEQUENZPROTOKOLL

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<120> DNA-Sequenz kodierend fuer eine
1-Deoxy-D-xylulose-5-phosphat Synthase

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Gly Leu Ser Thr Asp Ser Cys Lys Ser Thr Ser Leu Ser Ser Ser Arg
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     50                  55                  60

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Gln Ala Tyr Glu Ala Met Asn Asn Ala Gly Tyr Leu Asp Ser Asp Met
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Lys Asp Val Val Ala Ile His Ala Ala Met Gly Gly Gly Thr Gly Leu
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Gly Tyr Gly Ser Ala Val Gln Ser Cys Leu Gly Ala Ala Val Met Leu
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gaa gaa cgc gga tta aac gta act gta gcg gat gca cgg ttt tgc aag   1872
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Pro Leu Asp Arg Ala Leu Ile Arg Ser Leu Ala Lys Ser His Glu Val
625                 630                 635                 640

ctg atc acg gtt gaa gaa ggt tcc att gga ggt ttt ggc tcg cac gtt   1968
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Leu Lys Pro Phe Cys Ala Ile Tyr Ser Ser Phe Met Gln Arg Ala Tyr
465 470 475 480

Asp Gln Val Val His Asp Val Asp Leu Gln Lys Leu Pro Val Arg Phe
485 490 495

Ala Met Asp Arg Ala Gly Leu Val Gly Ala Asp Gly Pro Thr His Cys
500 505 510

Gly Ala Phe Asp Val Thr Phe Met Ala Cys Leu Pro Asn Met Ile Val
515 520 525

Met Ala Pro Ser Asp Glu Ala Asp Leu Phe Asn Met Val Ala Thr Ala
530 535 540

Val Ala Ile Asp Asp Arg Pro Ser Cys Phe Arg Tyr Pro Arg Gly Asn
545 550 555 560

Gly Ile Gly Val Ala Leu Pro Pro Gly Asn Lys Gly Val Pro Ile Glu
565 570 575

Ile Gly Lys Gly Arg Ile Leu Lys Glu Gly Glu Arg Val Ala Leu Leu
580 585 590

Gly Tyr Gly Ser Ala Val Gln Ser Cys Leu Gly Ala Ala Val Met Leu
595 600 605

Glu Glu Arg Gly Leu Asn Val Thr Val Ala Asp Ala Arg Phe Cys Lys
610 615 620

Pro Leu Asp Arg Ala Leu Ile Arg Ser Leu Ala Lys Ser His Glu Val
625 630 635 640

Leu Ile Thr Val Glu Glu Gly Ser Ile Gly Gly Phe Gly Ser His Val
645 650 655

Val Gln Phe Leu Ala Leu Asp Gly Leu Leu Asp Gly Lys Leu Lys Trp
660 665 670

Arg Pro Met Val Leu Pro Asp Arg Tyr Ile Asp His Gly Ala Pro Ala
675 680 685

Asp Gln Leu Ala Glu Ala Gly Leu Met Pro Ser His Ile Ala Ala Thr
690 695 700

Ala Leu Asn Leu Ile Gly Ala Pro Arg Glu Ala Leu Phe
705 710 715

<210> 3
<211> 1863
<212> DNA
<213> Escherichia coli

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Met Ser Phe Asp Ile Ala Lys Tyr Pro Thr Leu Ala Leu Val Asp Ser
1 5 10 15

acc cag gag tta cga ctg ttg ccg aaa gag agt tta ccg aaa ctc tgc 96
Thr Gln Glu Leu Arg Leu Leu Pro Lys Glu Ser Leu Pro Lys Leu Cys
20 25 30

gac gaa ctg cgc cgc tat tta ctc gac agc gtg agc cgt tcc agc ggg 144
Asp Glu Leu Arg Arg Tyr Leu Leu Asp Ser Val Ser Arg Ser Ser Gly
35 40 45

cac ttc gcc tcc ggg ctg ggc acg gtc gaa ctg acc gtg gcg ctg cac 192

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His Phe Ala Ser Gly Leu Gly Thr Val Glu Leu Thr Val Ala Leu His
 50                  55                  60

tat gtc tac aac acc ccg ttt gac caa ttg att tgg gat gtg ggg cat   240
Tyr Val Tyr Asn Thr Pro Phe Asp Gln Leu Ile Trp Asp Val Gly His
 65                  70                  75                  80

cag gct tat ccg cat aaa att ttg acc gga cgc cgc gac aaa atc ggc   288
Gln Ala Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Lys Ile Gly
                 85                  90                  95

acc atc cgt cag aaa ggc ggt ctg cac ccg ttc ccg tgg cgc ggc gaa   336
Thr Ile Arg Gln Lys Gly Gly Leu His Pro Phe Pro Trp Arg Gly Glu
            100                 105                 110

agc gaa tat gac gta tta agc gtc ggg cat tca tca acc tcc atc agt   384
Ser Glu Tyr Asp Val Leu Ser Val Gly His Ser Ser Thr Ser Ile Ser
        115                 120                 125

gcc gga att ggt att gcg gtt gct gcc gaa aaa gaa ggc aaa aat cgc   432
Ala Gly Ile Gly Ile Ala Val Ala Ala Glu Lys Glu Gly Lys Asn Arg
    130                 135                 140

cgc acc gtc tgt gtc att ggc gat ggc gcg att acc gca ggc atg gcg   480
Arg Thr Val Cys Val Ile Gly Asp Gly Ala Ile Thr Ala Gly Met Ala
145                 150                 155                 160

ttt gaa gcg atg aat cac gcg ggc gat atc cgt cct gat atg ctg gtg   528
Phe Glu Ala Met Asn His Ala Gly Asp Ile Arg Pro Asp Met Leu Val
                165                 170                 175

att ctc aac gac aat gaa atg tcg att tcc gaa aat gtc ggc gcg ctc   576
Ile Leu Asn Asp Asn Glu Met Ser Ile Ser Glu Asn Val Gly Ala Leu
            180                 185                 190

aac aac cat ctg gca cag ctg ctt tcc ggt aag ctt tac tct tca ctg   624
Asn Asn His Leu Ala Gln Leu Leu Ser Gly Lys Leu Tyr Ser Ser Leu
        195                 200                 205

cgc gaa ggc ggg aaa aaa gtt ttc tct ggc gtg ccg cca att aaa gag   672
Arg Glu Gly Gly Lys Lys Val Phe Ser Gly Val Pro Pro Ile Lys Glu
    210                 215                 220

ctg ctc aaa cgc acc gaa gaa cat att aaa ggc atg gta gtg cct ggc   720
Leu Leu Lys Arg Thr Glu Glu His Ile Lys Gly Met Val Val Pro Gly
225                 230                 235                 240

acg ttg ttt gaa gag ctg ggc ttt aac tac atc ggc ccg gtg gac ggt   768
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```
Thr Leu Phe Glu Glu Leu Gly Phe Asn Tyr Ile Gly Pro Val Asp Gly
                245                 250                 255

cac gat gtg ctg ggg ctt atc acc acg cta aag aac atg cgc gac ctg   816
His Asp Val Leu Gly Leu Ile Thr Thr Leu Lys Asn Met Arg Asp Leu
            260                 265                 270

aaa ggc ccg cag ttc ctg cat atc atg acc aaa aaa ggt cgt ggt tat   864
Lys Gly Pro Gln Phe Leu His Ile Met Thr Lys Lys Gly Arg Gly Tyr
        275                 280                 285

gaa ccg gca gaa aaa gac ccg atc act ttc cac gcc gtg cct aaa ttt   912
Glu Pro Ala Glu Lys Asp Pro Ile Thr Phe His Ala Val Pro Lys Phe
    290                 295                 300

gat ccc tcc agc ggt tgt ttg ccg aaa agt agc ggc ggt ttg ccg agc   960
Asp Pro Ser Ser Gly Cys Leu Pro Lys Ser Ser Gly Gly Leu Pro Ser
305                 310                 315                 320

tat tca aaa atc ttt ggc gac tgg ttg tgc gaa acg gca gcg aaa gac  1008
Tyr Ser Lys Ile Phe Gly Asp Trp Leu Cys Glu Thr Ala Ala Lys Asp
                325                 330                 335

aac aag ctg atg gcg att act ccg gcg atg cgt gaa ggt tcc ggc atg  1056
Asn Lys Leu Met Ala Ile Thr Pro Ala Met Arg Glu Gly Ser Gly Met
            340                 345                 350

gtc gag ttt tca cgt aaa ttc ccg gat cgc tac ttc gac gtg gca att  1104
Val Glu Phe Ser Arg Lys Phe Pro Asp Arg Tyr Phe Asp Val Ala Ile
        355                 360                 365

gcc gag caa cac gcg gtg acc ttt gct gcg ggt ctg gcg att ggt ggg  1152
Ala Glu Gln His Ala Val Thr Phe Ala Ala Gly Leu Ala Ile Gly Gly
    370                 375                 380

tac aaa ccc att gtc gcg att tac tcc act ttc ctg caa cgc gcc tat  1200
Tyr Lys Pro Ile Val Ala Ile Tyr Ser Thr Phe Leu Gln Arg Ala Tyr
385                 390                 395                 400

gat cag gtg ctg cat gac gtg gcg att caa aag ctt ccg gtc ctg ttc  1248
Asp Gln Val Leu His Asp Val Ala Ile Gln Lys Leu Pro Val Leu Phe
                405                 410                 415

gcc atc gac cgc gcg ggc att gtt ggt gct gac ggt caa acc cat cag  1296
Ala Ile Asp Arg Ala Gly Ile Val Gly Ala Asp Gly Gln Thr His Gln
            420                 425                 430

ggt gct ttt gat ctc tct tac ctg cgc tgc ata ccg gaa atg gtc att  1344
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Gly Ala Phe Asp Leu Ser Tyr Leu Arg Cys Ile Pro Glu Met Val Ile
        435                 440                 445

atg acc ccg agc gat gaa aac gaa tgt cgc cag atg ctc tat acc ggc   1392
Met Thr Pro Ser Asp Glu Asn Glu Cys Arg Gln Met Leu Tyr Thr Gly
    450                 455                 460

tat cac tat aac gat ggc ccg tca gcg gtg cgc tac ccg cgt ggc aac   1440
Tyr His Tyr Asn Asp Gly Pro Ser Ala Val Arg Tyr Pro Arg Gly Asn
465                 470                 475                 480

gcg gtc ggc gtg gaa ctg acg ccg ctg gaa aaa cta cca att ggc aaa   1488
Ala Val Gly Val Glu Leu Thr Pro Leu Glu Lys Leu Pro Ile Gly Lys
                485                 490                 495

ggc att gtg aag cgt cgt ggc gag aaa ctg gcg atc ctt aac ttt ggt   1536
Gly Ile Val Lys Arg Arg Gly Glu Lys Leu Ala Ile Leu Asn Phe Gly
            500                 505                 510

acg ctg atg cca gaa gcg gcg aaa gtc gcc gaa tcg ctg aac gcc acg   1584
Thr Leu Met Pro Glu Ala Ala Lys Val Ala Glu Ser Leu Asn Ala Thr
        515                 520                 525

ctg gtc gat atg cgt ttt gtg aaa ccg ctt gat gaa gcg tta att ctg   1632
Leu Val Asp Met Arg Phe Val Lys Pro Leu Asp Glu Ala Leu Ile Leu
    530                 535                 540

gaa atg gcc gcc agc cat gaa gcg ctg gtc acc gta gaa gaa aac gcc   1680
Glu Met Ala Ala Ser His Glu Ala Leu Val Thr Val Glu Glu Asn Ala
545                 550                 555                 560

att atg ggc ggc gca ggc agc ggc gtg aac gaa gtg ctg atg gcc cat   1728
Ile Met Gly Gly Ala Gly Ser Gly Val Asn Glu Val Leu Met Ala His
                565                 570                 575

cgt aaa cca gta ccc gtg ctg aac att ggc ctg ccg gac ttc ttt att   1776
Arg Lys Pro Val Pro Val Leu Asn Ile Gly Leu Pro Asp Phe Phe Ile
            580                 585                 590

ccg caa gga act cag gaa gaa atg cgc gcc gaa ctc ggc ctc gat gcc   1824
Pro Gln Gly Thr Gln Glu Glu Met Arg Ala Glu Leu Gly Leu Asp Ala
        595                 600                 605

gct ggt atg gaa gcc aaa atc aag gcc tgg ctg gca taa               1863
Ala Gly Met Glu Ala Lys Ile Lys Ala Trp Leu Ala
    610                 615                 620
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<210> 4
<211> 620
<212> PRT
<213> Escherichia coli

<400> 4

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Met Ser Phe Asp Ile Ala Lys Tyr Pro Thr Leu Ala Leu Val Asp Ser
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Thr Gln Glu Leu Arg Leu Leu Pro Lys Glu Ser Leu Pro Lys Leu Cys
            20                  25                  30

Asp Glu Leu Arg Arg Tyr Leu Leu Asp Ser Val Ser Arg Ser Ser Gly
        35                  40                  45

His Phe Ala Ser Gly Leu Gly Thr Val Glu Leu Thr Val Ala Leu His
    50                  55                  60

Tyr Val Tyr Asn Thr Pro Phe Asp Gln Leu Ile Trp Asp Val Gly His
65                  70                  75                  80

Gln Ala Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Lys Ile Gly
                85                  90                  95

Thr Ile Arg Gln Lys Gly Gly Leu His Pro Phe Pro Trp Arg Gly Glu
            100                 105                 110

Ser Glu Tyr Asp Val Leu Ser Val Gly His Ser Ser Thr Ser Ile Ser
        115                 120                 125

Ala Gly Ile Gly Ile Ala Val Ala Ala Glu Lys Glu Gly Lys Asn Arg
    130                 135                 140

Arg Thr Val Cys Val Ile Gly Asp Gly Ala Ile Thr Ala Gly Met Ala
145                 150                 155                 160

Phe Glu Ala Met Asn His Ala Gly Asp Ile Arg Pro Asp Met Leu Val
                165                 170                 175

Ile Leu Asn Asp Asn Glu Met Ser Ile Ser Glu Asn Val Gly Ala Leu
            180                 185                 190

Asn Asn His Leu Ala Gln Leu Leu Ser Gly Lys Leu Tyr Ser Ser Leu
        195                 200                 205

Arg Glu Gly Gly Lys Lys Val Phe Ser Gly Val Pro Pro Ile Lys Glu
    210                 215                 220
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Leu Leu Lys Arg Thr Glu Glu His Ile Lys Gly Met Val Val Pro Gly
225 230 235 240

Thr Leu Phe Glu Glu Leu Gly Phe Asn Tyr Ile Gly Pro Val Asp Gly
245 250 255

His Asp Val Leu Gly Leu Ile Thr Thr Leu Lys Asn Met Arg Asp Leu
260 265 270

Lys Gly Pro Gln Phe Leu His Ile Met Thr Lys Lys Gly Arg Gly Tyr
275 280 285

Glu Pro Ala Glu Lys Asp Pro Ile Thr Phe His Ala Val Pro Lys Phe
290 295 300

Asp Pro Ser Ser Gly Cys Leu Pro Lys Ser Ser Gly Gly Leu Pro Ser
305 310 315 320

Tyr Ser Lys Ile Phe Gly Asp Trp Leu Cys Glu Thr Ala Ala Lys Asp
325 330 335

Asn Lys Leu Met Ala Ile Thr Pro Ala Met Arg Glu Gly Ser Gly Met
340 345 350

Val Glu Phe Ser Arg Lys Phe Pro Asp Arg Tyr Phe Asp Val Ala Ile
355 360 365

Ala Glu Gln His Ala Val Thr Phe Ala Ala Gly Leu Ala Ile Gly Gly
370 375 380

Tyr Lys Pro Ile Val Ala Ile Tyr Ser Thr Phe Leu Gln Arg Ala Tyr
385 390 395 400

Asp Gln Val Leu His Asp Val Ala Ile Gln Lys Leu Pro Val Leu Phe
405 410 415

Ala Ile Asp Arg Ala Gly Ile Val Gly Ala Asp Gly Gln Thr His Gln
420 425 430

Gly Ala Phe Asp Leu Ser Tyr Leu Arg Cys Ile Pro Glu Met Val Ile
435 440 445

Met Thr Pro Ser Asp Glu Asn Glu Cys Arg Gln Met Leu Tyr Thr Gly
450 455 460

Tyr His Tyr Asn Asp Gly Pro Ser Ala Val Arg Tyr Pro Arg Gly Asn
465 470 475 480

Ala Val Gly Val Glu Leu Thr Pro Leu Glu Lys Leu Pro Ile Gly Lys
485 490 495

Gly Ile Val Lys Arg Arg Gly Glu Lys Leu Ala Ile Leu Asn Phe Gly
500 505 510

Thr Leu Met Pro Glu Ala Ala Lys Val Ala Glu Ser Leu Asn Ala Thr
515 520 525

Leu Val Asp Met Arg Phe Val Lys Pro Leu Asp Glu Ala Leu Ile Leu
530 535 540

Glu Met Ala Ala Ser His Glu Ala Leu Val Thr Val Glu Glu Asn Ala
545 550 555 560

Ile Met Gly Gly Ala Gly Ser Gly Val Asn Glu Val Leu Met Ala His
565 570 575

Arg Lys Pro Val Pro Val Leu Asn Ile Gly Leu Pro Asp Phe Phe Ile
580 585 590

Pro Gln Gly Thr Gln Glu Glu Met Arg Ala Glu Leu Gly Leu Asp Ala
595 600 605

Ala Gly Met Glu Ala Lys Ile Lys Ala Trp Leu Ala
610 615 620

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<213> Streptomyces avermitilis

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<222> (218)..(1138)

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gagcactccg atgcgcggct cccgcgccag cagcaccagg agccggccgt ccagatgatc 180

gatcgccacg gcagcccctc cagtggtcat cctgtac atg cag ccc cac gcc atg 235
Met Gln Pro His Ala Met
1 5

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ggc ggt gca ctg aac aca ttg tcc agc gga caa gcc aac tat tgc gca   283
Gly Gly Ala Leu Asn Thr Leu Ser Ser Gly Gln Ala Asn Tyr Cys Ala
            10                  15                  20

cct tgc gga acg gag cga ccc tgc cgc cat gac gca gac cac aca cca   331
Pro Cys Gly Thr Glu Arg Pro Cys Arg His Asp Ala Asp His Thr Pro
        25                  30                  35

cac tcc cga cac cgc ccg gca ggc cga ccc ctt ccc ggt gaa ggg aat   379
His Ser Arg His Arg Pro Ala Gly Arg Pro Leu Pro Gly Glu Gly Asn
    40                  45                  50

gga cgc ggt cgt ctt cgc cgt agg caa cgc caa gca ggc cgc gca cta   427
Gly Arg Gly Arg Leu Arg Arg Arg Gln Arg Gln Ala Gly Arg Ala Leu
55                  60                  65                  70

ctc cac cgc ctt cgg cat gca gct tgt ggc gta ctc cgg acc gga gaa   475
Leu His Arg Leu Arg His Ala Ala Cys Gly Val Leu Arg Thr Gly Glu
                75                  80                  85

cgg cag ccg cga gac cgc ttc gta cgt cct cac caa cgg ctc ggc acg   523
Arg Gln Pro Arg Asp Arg Phe Val Arg Pro His Gln Arg Leu Gly Thr
            90                  95                  100

ctt cgt cct cac ctc cgt cat caa gcc cgc cac ccc ctg ggg cca ctt   571
Leu Arg Pro His Leu Arg His Gln Ala Arg His Pro Leu Gly Pro Leu
        105                 110                 115

cct cgc cga cca tgt ggc cga gca cgg cga cgg cgt cgt cga cct cgc   619
Pro Arg Arg Pro Cys Gly Arg Ala Arg Arg Arg Arg Arg Arg Pro Arg
    120                 125                 130

cat cga ggt ccc gga cgc ccg cgc cgc cca cgc gta cgc gat cga gca   667
His Arg Gly Pro Gly Arg Pro Arg Arg Pro Arg Val Arg Asp Arg Ala
135                 140                 145                 150

cgg cgc ccg ctc ggt cgc cga gcc gta cga gct gaa gga cga gca cgg   715
Arg Arg Pro Leu Gly Arg Arg Ala Val Arg Ala Glu Gly Arg Ala Arg
                155                 160                 165

cac ggt cgt cct cgc cgc gat cgc cac cta cgg caa gac ccg cca cac   763
His Gly Arg Pro Arg Arg Asp Arg His Leu Arg Gln Asp Pro Pro His
            170                 175                 180

cct cgt cga ccg gac cgg cta cga cgg ccc cta cct ccc cgg cta cgt   811
Pro Arg Arg Pro Asp Arg Leu Arg Arg Pro Leu Pro Pro Arg Leu Arg
        185                 190                 195
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ggc cgc cgc ccc gat cgt cga acc gcc cgc cca ccg cac ctt cca ggc   859
Gly Arg Arg Pro Asp Arg Arg Thr Ala Arg Pro Pro His Leu Pro Gly
    200                 205                 210

cat cga cca ctg cgt cgg caa cgt cga gct cgg ccg gat gaa cga atg   907
His Arg Pro Leu Arg Arg Gln Arg Arg Ala Arg Pro Asp Glu Arg Met
215                 220                 225                 230

ggt cgg ctt cta caa caa ggt cat ggg ctt cac gaa cat gaa gga gtt   955
Gly Arg Leu Leu Gln Gln Gly His Gly Leu His Glu His Glu Gly Val
                235                 240                 245

cgt ggg cga cga cat cgc gac cga gta ctc ggc gct gat gtc gaa ggt   1003
Arg Gly Arg Arg His Arg Asp Arg Val Leu Gly Ala Asp Val Glu Gly
            250                 255                 260

cgt ggc cga cgg cac gct caa ggt caa gtt ccc gat caa cga gcc cgc   1051
Arg Gly Arg Arg His Ala Gln Gly Gln Val Pro Asp Gln Arg Ala Arg
        265                 270                 275

cct cgc caa gaa gaa gtc cca gat cga cga gta cct gga gtt cta cgg   1099
Pro Arg Gln Glu Glu Val Pro Asp Arg Arg Val Pro Gly Val Leu Arg
    280                 285                 290

cgg cgc ggg cgt cca gca cat cgc gct gaa cac ggg tga catcgtcgag    1148
Arg Arg Gly Arg Pro Ala His Arg Ala Glu His Gly
295                 300                 305

acggtacgca cgatgcgcgc cgccggcgtc cagttcctgg acacgcccga ctcgtactac 1208

gacaccctcg gggagtgggt gggcgacacc cgcgtccccg tcgacaccct gcgcgagctg 1268

aagatcctcg cggaccgcga cgaggacggc tatctgctcc agatcttcac caagccggtc 1328

caggaccgcc cgacggtctt cttcgagatc atcgaacgcc acggctcgat gggattcggc 1388

aagggcaact tcaaggccct gttcgaggcg atcgagcggg agcaggagaa gcggggcaac 1448

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<212> PRT
<213> Streptomyces avermitilis

<400> 6
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Gln Ala Asn Tyr Cys Ala Pro Cys Gly Thr Glu Arg Pro Cys Arg His
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Asp Ala Asp His Thr Pro His Ser Arg His Arg Pro Ala Gly Arg Pro
35 40 45

Leu Pro Gly Glu Gly Asn Gly Arg Gly Arg Leu Arg Arg Arg Gln Arg
50 55 60

Gln Ala Gly Arg Ala Leu Leu His Arg Leu Arg His Ala Ala Cys Gly
65 70 75 80

Val Leu Arg Thr Gly Glu Arg Gln Pro Arg Asp Arg Phe Val Arg Pro
85 90 95

His Gln Arg Leu Gly Thr Leu Arg Pro His Leu Arg His Gln Ala Arg
100 105 110

His Pro Leu Gly Pro Leu Pro Arg Arg Pro Cys Gly Arg Ala Arg Arg
115 120 125

Arg Arg Arg Arg Pro Arg His Arg Gly Pro Gly Arg Pro Arg Arg Pro
130 135 140

Arg Val Arg Asp Arg Ala Arg Arg Pro Leu Gly Arg Arg Ala Val Arg
145 150 155 160

Ala Glu Gly Arg Ala Arg His Gly Arg Pro Arg Arg Asp Arg His Leu
165 170 175

Arg Gln Asp Pro Pro His Pro Arg Arg Pro Asp Arg Leu Arg Arg Pro
180 185 190

Leu Pro Pro Arg Leu Arg Gly Arg Arg Pro Asp Arg Arg Thr Ala Arg
195 200 205

Pro Pro His Leu Pro Gly His Arg Pro Leu Arg Arg Gln Arg Arg Ala
210 215 220

Arg Pro Asp Glu Arg Met Gly Arg Leu Leu Gln Gln Gly His Gly Leu
225 230 235 240

His Glu His Glu Gly Val Arg Gly Arg Arg His Arg Asp Arg Val Leu
245 250 255

Gly Ala Asp Val Glu Gly Arg Gly Arg Arg His Ala Gln Gly Gln Val

260 265 270

Pro Asp Gln Arg Ala Arg Pro Arg Gln Glu Glu Val Pro Asp Arg Arg
275 280 285

Val Pro Gly Val Leu Arg Arg Arg Gly Arg Pro Ala His Arg Ala Glu
290 295 300

His Gly
305

<210> 7
<211> 1479
<212> DNA
<213> Arabidopsis thaliana

<220>
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<222> (1)..(1401)

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1 5 10 15

tca acg gag caa aca aac ttc gtc tct cat gta ccg tca tca ctt tct 96
Ser Thr Glu Gln Thr Asn Phe Val Ser His Val Pro Ser Ser Leu Ser
20 25 30

ctc cct caa cga cgg acc tct ctc cga gta acc gca gcc agg gcc act 144
Leu Pro Gln Arg Arg Thr Ser Leu Arg Val Thr Ala Ala Arg Ala Thr
35 40 45

ccc aaa ctc tcc aac cgt aaa ctc cgt gtc gcc gtc atc ggt ggt gga 192
Pro Lys Leu Ser Asn Arg Lys Leu Arg Val Ala Val Ile Gly Gly Gly
50 55 60

cca gca ggc ggg gca gct gca gag act cta gca caa gga gga atc gag 240
Pro Ala Gly Gly Ala Ala Ala Glu Thr Leu Ala Gln Gly Gly Ile Glu
65 70 75 80

acg att ctc atc gag cgt aag atg gac aat tgc aag cct tgc ggt ggc 288
Thr Ile Leu Ile Glu Arg Lys Met Asp Asn Cys Lys Pro Cys Gly Gly
85 90 95

gcg att cct ctc tgt atg gtc gga gaa ttc aac ttg ccg ttg gat att 336
Ala Ile Pro Leu Cys Met Val Gly Glu Phe Asn Leu Pro Leu Asp Ile

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                100                 105                 110

att gat cgg aga gtg acg aag atg aag atg att tcg ccg tcg aac att   384
Ile Asp Arg Arg Val Thr Lys Met Lys Met Ile Ser Pro Ser Asn Ile
        115                 120                 125

gct gtt gat att ggt cgt acg ctt aag gag cat gag tat ata ggt atg   432
Ala Val Asp Ile Gly Arg Thr Leu Lys Glu His Glu Tyr Ile Gly Met
    130                 135                 140

gtg aga aga gaa gtt ctt gat gct tat ctg aga gag aga gct gag aag   480
Val Arg Arg Glu Val Leu Asp Ala Tyr Leu Arg Glu Arg Ala Glu Lys
145                 150                 155                 160

agt gga gcc act gtg att aac ggt ctc ttc ctt aag atg gat cat ccg   528
Ser Gly Ala Thr Val Ile Asn Gly Leu Phe Leu Lys Met Asp His Pro
                165                 170                 175

gag aat tgg gac tcg ccg tac act ttg cat tac act gag tac gat ggt   576
Glu Asn Trp Asp Ser Pro Tyr Thr Leu His Tyr Thr Glu Tyr Asp Gly
            180                 185                 190

aaa act gga gct aca ggg acg aag aaa aca atg gag gtt gat gct gtc   624
Lys Thr Gly Ala Thr Gly Thr Lys Lys Thr Met Glu Val Asp Ala Val
        195                 200                 205

att gga gct gat gga gct aac tct agg gtt gct aaa tct att gat gct   672
Ile Gly Ala Asp Gly Ala Asn Ser Arg Val Ala Lys Ser Ile Asp Ala
    210                 215                 220

ggt gat tac gac tac gca att gca ttt cag gag agg att agg att cct   720
Gly Asp Tyr Asp Tyr Ala Ile Ala Phe Gln Glu Arg Ile Arg Ile Pro
225                 230                 235                 240

gat gag aaa atg act tac tat gag gat tta gct gag atg tat gtt gga   768
Asp Glu Lys Met Thr Tyr Tyr Glu Asp Leu Ala Glu Met Tyr Val Gly
                245                 250                 255

gat gat gtg tcg ccg gat ttc tat ggt tgg gtg ttc cct aag tgc gac   816
Asp Asp Val Ser Pro Asp Phe Tyr Gly Trp Val Phe Pro Lys Cys Asp
            260                 265                 270

cat gta gct gtt gga aca ggt act gtg act cac aaa ggt gac atc aag   864
His Val Ala Val Gly Thr Gly Thr Val Thr His Lys Gly Asp Ile Lys
        275                 280                 285

aag ttc cag ctc gcg acc aga aac aga gct aag gac aag att ctt gga   912
Lys Phe Gln Leu Ala Thr Arg Asn Arg Ala Lys Asp Lys Ile Leu Gly
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  290                 295                 300

ggg aag atc atc cgt gtg gag gct cat ccg att cct gaa cat ccg aga   960
Gly Lys Ile Ile Arg Val Glu Ala His Pro Ile Pro Glu His Pro Arg
305                 310                 315                 320

cca cgt agg ctc tcg aaa cgt gtg gct ctt gta ggt gat gct gca ggg   1008
Pro Arg Arg Leu Ser Lys Arg Val Ala Leu Val Gly Asp Ala Ala Gly
                325                 330                 335

tat gtg act aaa tgc tct ggt gaa ggg atc tac ttt gct gct aag agt   1056
Tyr Val Thr Lys Cys Ser Gly Glu Gly Ile Tyr Phe Ala Ala Lys Ser
            340                 345                 350

gga aga atg tgt gct gaa gcc att gtc gaa ggt tca cag aat ggt aag   1104
Gly Arg Met Cys Ala Glu Ala Ile Val Glu Gly Ser Gln Asn Gly Lys
        355                 360                 365

aag atg att gac gaa ggg gac ttg agg aag tac ttg gag aaa tgg gat   1152
Lys Met Ile Asp Glu Gly Asp Leu Arg Lys Tyr Leu Glu Lys Trp Asp
    370                 375                 380

aag aca tac ttg cct acc tac agg gta ctt gat gtg ttg cag aaa gtg   1200
Lys Thr Tyr Leu Pro Thr Tyr Arg Val Leu Asp Val Leu Gln Lys Val
385                 390                 395                 400

ttt tac aga tca aat ccg gct aga gaa gcg ttt gtg gag atg tgt aat   1248
Phe Tyr Arg Ser Asn Pro Ala Arg Glu Ala Phe Val Glu Met Cys Asn
                405                 410                 415

gat gag tat gtt cag aag atg aca ttc gat agc tat ctg tac aag cgg   1296
Asp Glu Tyr Val Gln Lys Met Thr Phe Asp Ser Tyr Leu Tyr Lys Arg
            420                 425                 430

gtt gcg ccg ggt agt cct ttg gag gat atc aag ttg gct gtg aac acc   1344
Val Ala Pro Gly Ser Pro Leu Glu Asp Ile Lys Leu Ala Val Asn Thr
        435                 440                 445

att gga agt ttg gtt agg gct aat gct cta agg aga gag att gag aag   1392
Ile Gly Ser Leu Val Arg Ala Asn Ala Leu Arg Arg Glu Ile Glu Lys
    450                 455                 460

ctt agt gtt taagaaacaa ataatgaggt ctatctcctt tcttcatctc           1441
Leu Ser Val
465

tatctctctt tttttgtctg ttagtaatct atctacac                         1479
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Ser Thr Glu Gln Thr Asn Phe Val Ser His Val Pro Ser Ser Leu Ser
            20                  25                  30

Leu Pro Gln Arg Arg Thr Ser Leu Arg Val Thr Ala Ala Arg Ala Thr
        35                  40                  45

Pro Lys Leu Ser Asn Arg Lys Leu Arg Val Ala Val Ile Gly Gly Gly
    50                  55                  60

Pro Ala Gly Gly Ala Ala Ala Glu Thr Leu Ala Gln Gly Gly Ile Glu
65                  70                  75                  80

Thr Ile Leu Ile Glu Arg Lys Met Asp Asn Cys Lys Pro Cys Gly Gly
                85                  90                  95

Ala Ile Pro Leu Cys Met Val Gly Glu Phe Asn Leu Pro Leu Asp Ile
            100                 105                 110

Ile Asp Arg Arg Val Thr Lys Met Lys Met Ile Ser Pro Ser Asn Ile
        115                 120                 125

Ala Val Asp Ile Gly Arg Thr Leu Lys Glu His Glu Tyr Ile Gly Met
    130                 135                 140

Val Arg Arg Glu Val Leu Asp Ala Tyr Leu Arg Glu Arg Ala Glu Lys
145                 150                 155                 160

Ser Gly Ala Thr Val Ile Asn Gly Leu Phe Leu Lys Met Asp His Pro
                165                 170                 175

Glu Asn Trp Asp Ser Pro Tyr Thr Leu His Tyr Thr Glu Tyr Asp Gly
            180                 185                 190

Lys Thr Gly Ala Thr Gly Thr Lys Lys Thr Met Glu Val Asp Ala Val
        195                 200                 205

Ile Gly Ala Asp Gly Ala Asn Ser Arg Val Ala Lys Ser Ile Asp Ala
    210                 215                 220
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Gly Asp Tyr Asp Tyr Ala Ile Ala Phe Gln Glu Arg Ile Arg Ile Pro
225 230 235 240

Asp Glu Lys Met Thr Tyr Tyr Glu Asp Leu Ala Glu Met Tyr Val Gly
245 250 255

Asp Asp Val Ser Pro Asp Phe Tyr Gly Trp Val Phe Pro Lys Cys Asp
260 265 270

His Val Ala Val Gly Thr Gly Thr Val Thr His Lys Gly Asp Ile Lys
275 280 285

Lys Phe Gln Leu Ala Thr Arg Asn Arg Ala Lys Asp Lys Ile Leu Gly
290 295 300

Gly Lys Ile Ile Arg Val Glu Ala His Pro Ile Pro Glu His Pro Arg
305 310 315 320

Pro Arg Arg Leu Ser Lys Arg Val Ala Leu Val Gly Asp Ala Ala Gly
325 330 335

Tyr Val Thr Lys Cys Ser Gly Glu Gly Ile Tyr Phe Ala Ala Lys Ser
340 345 350

Gly Arg Met Cys Ala Glu Ala Ile Val Glu Gly Ser Gln Asn Gly Lys
355 360 365

Lys Met Ile Asp Glu Gly Asp Leu Arg Lys Tyr Leu Glu Lys Trp Asp
370 375 380

Lys Thr Tyr Leu Pro Thr Tyr Arg Val Leu Asp Val Leu Gln Lys Val
385 390 395 400

Phe Tyr Arg Ser Asn Pro Ala Arg Glu Ala Phe Val Glu Met Cys Asn
405 410 415

Asp Glu Tyr Val Gln Lys Met Thr Phe Asp Ser Tyr Leu Tyr Lys Arg
420 425 430

Val Ala Pro Gly Ser Pro Leu Glu Asp Ile Lys Leu Ala Val Asn Thr
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Ile Gly Ser Leu Val Arg Ala Asn Ala Leu Arg Arg Glu Ile Glu Lys
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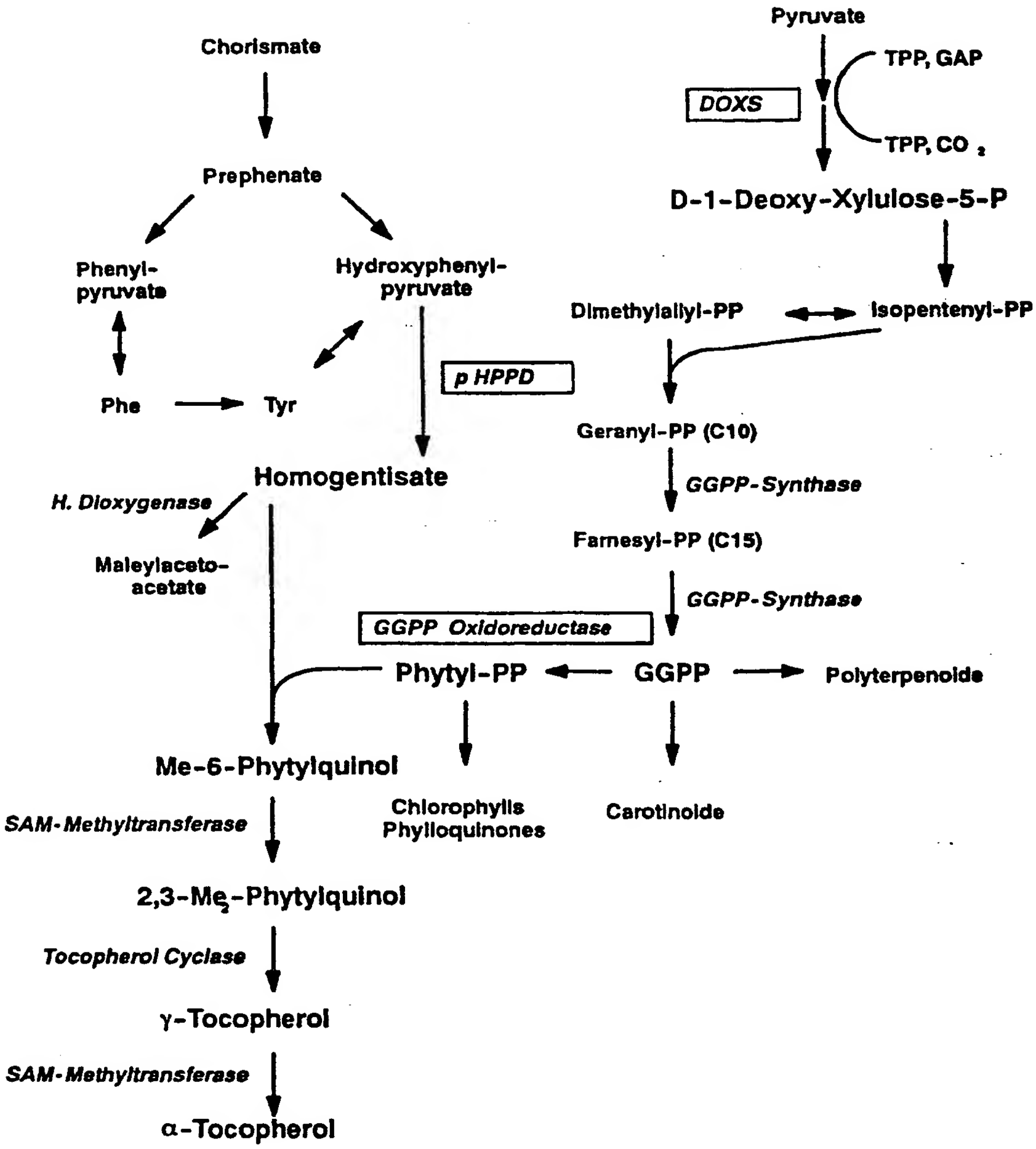
Leu Ser Val
465

0817/00006

1/NO TAG

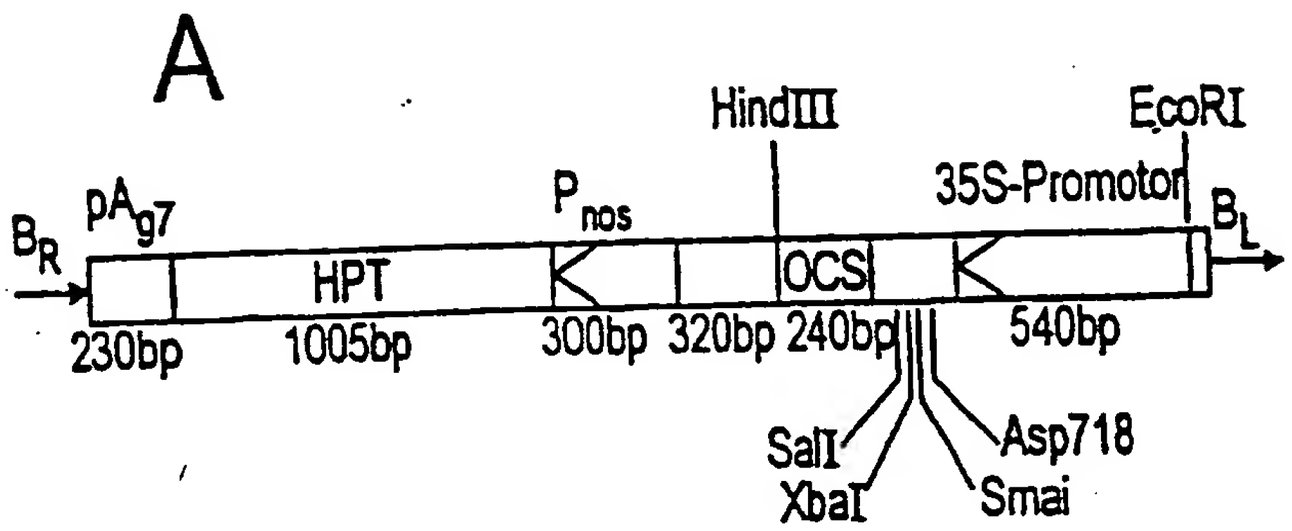
Figure 1

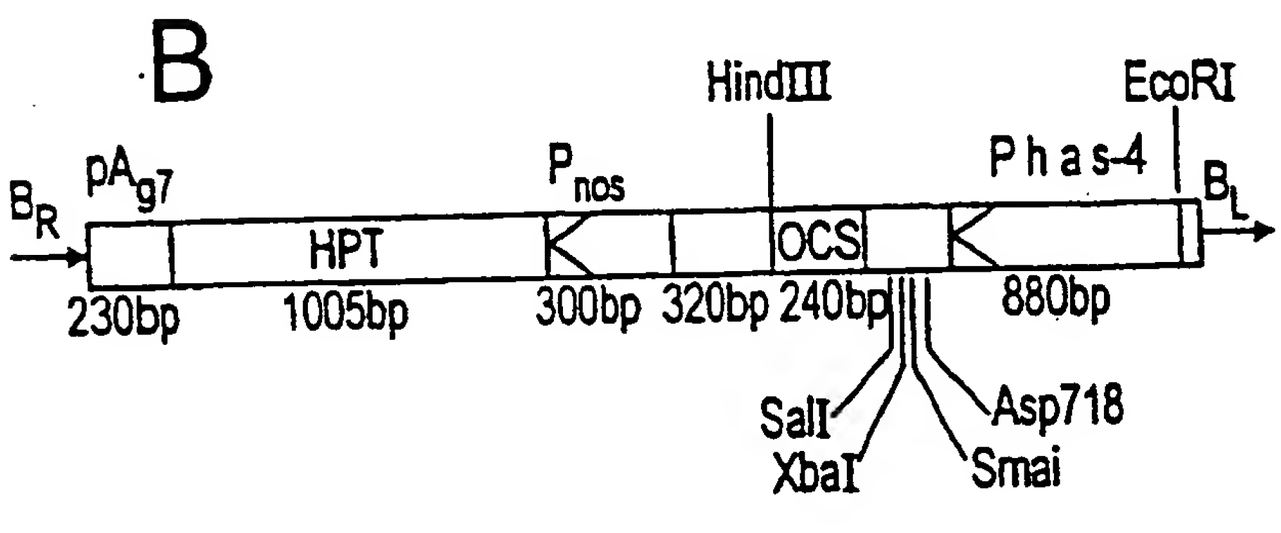


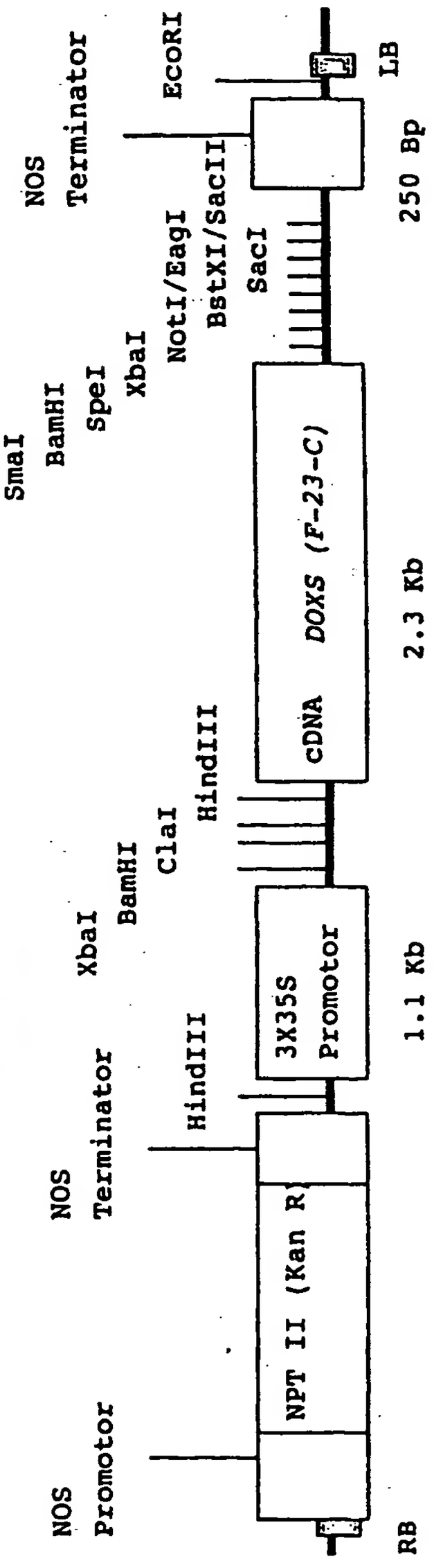
0817/00006

2/NO TAG

Figure 2



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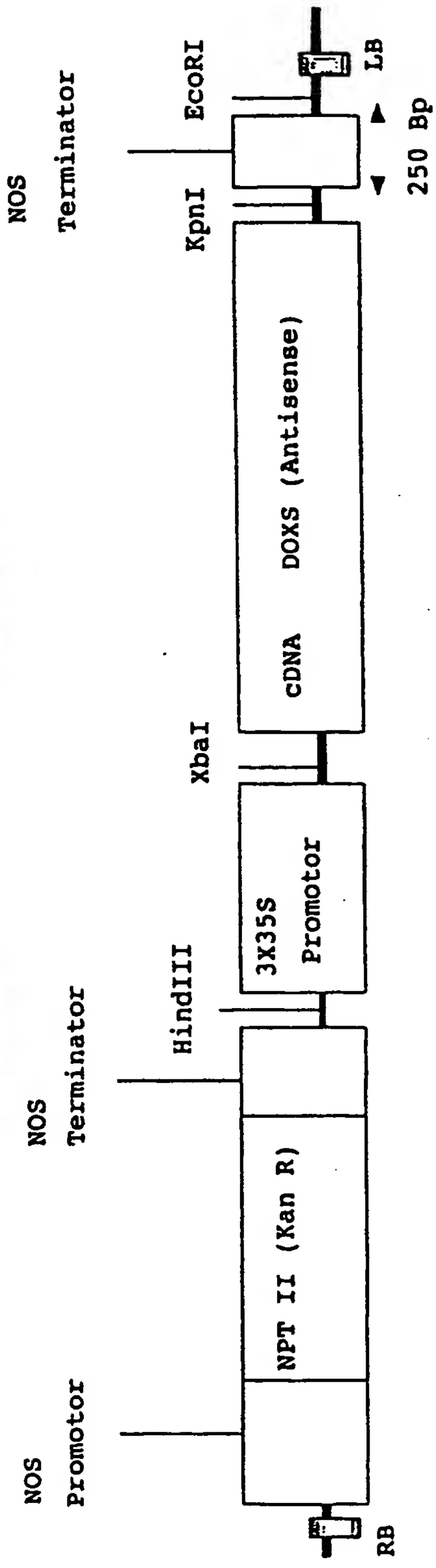
EcoRI
NotI/EagI
BstXI/SacII
SacI
LB
250 Bp
EcoRI
SmaI
BamHI
SpeI
XbaI
cDNA DOXS (F-23-C)
2.3 Kb
XbaI
BamHI
ClaI
HindIII
HindIII
3X35S Promotor
1.1 Kb
NOS Terminator
NPT II (Kan R)
NOS Promotor
RB

Figure 3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NOS
Terminator
HindIII
3X35S
Promotor
XbaI
cDNA DOXS (Antisense)
NOS
Promotor
NPT II (Kan R)
RB
NOS
Terminator
KpnI
EcoRI
LB
250 Bp

Figure 4

1/NO TAG

Figure 5

Binary vector for overexpression of the DOXS gene from E. coli in the cytosol of transgenic plants

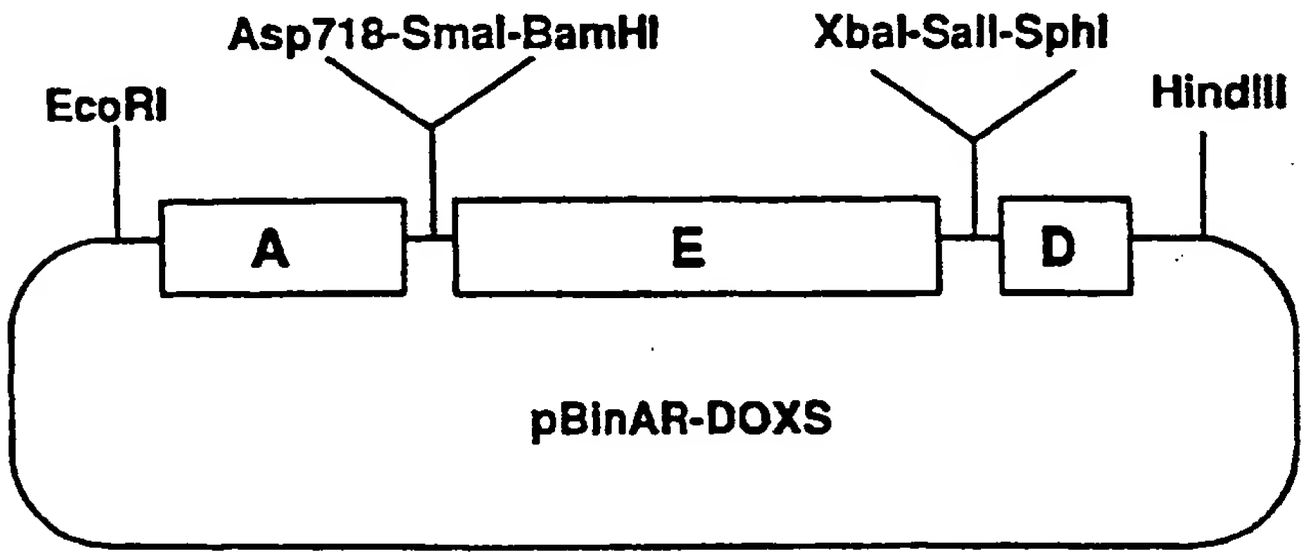
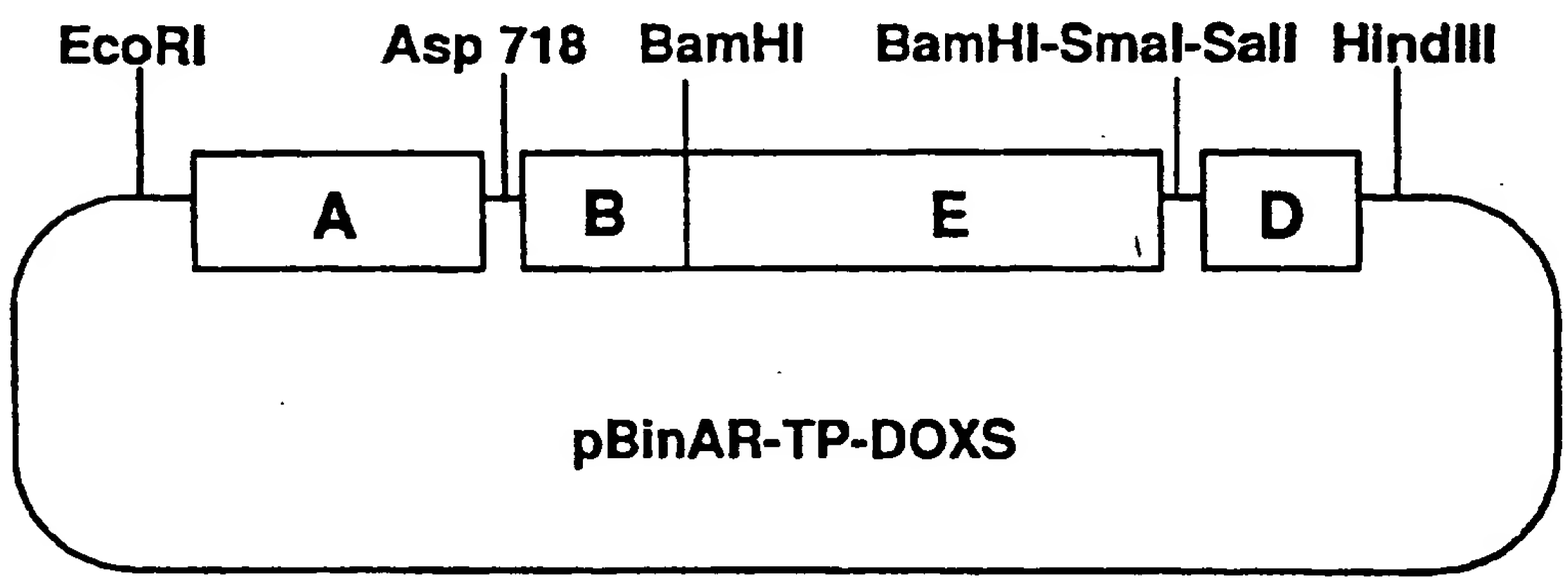


Figure 6

Binary vector for overexpression of the DOXS gene from E.coli in plastids of transgenic plants.



2/NO TAG

Figure 7: DOXS gene RNA expression level

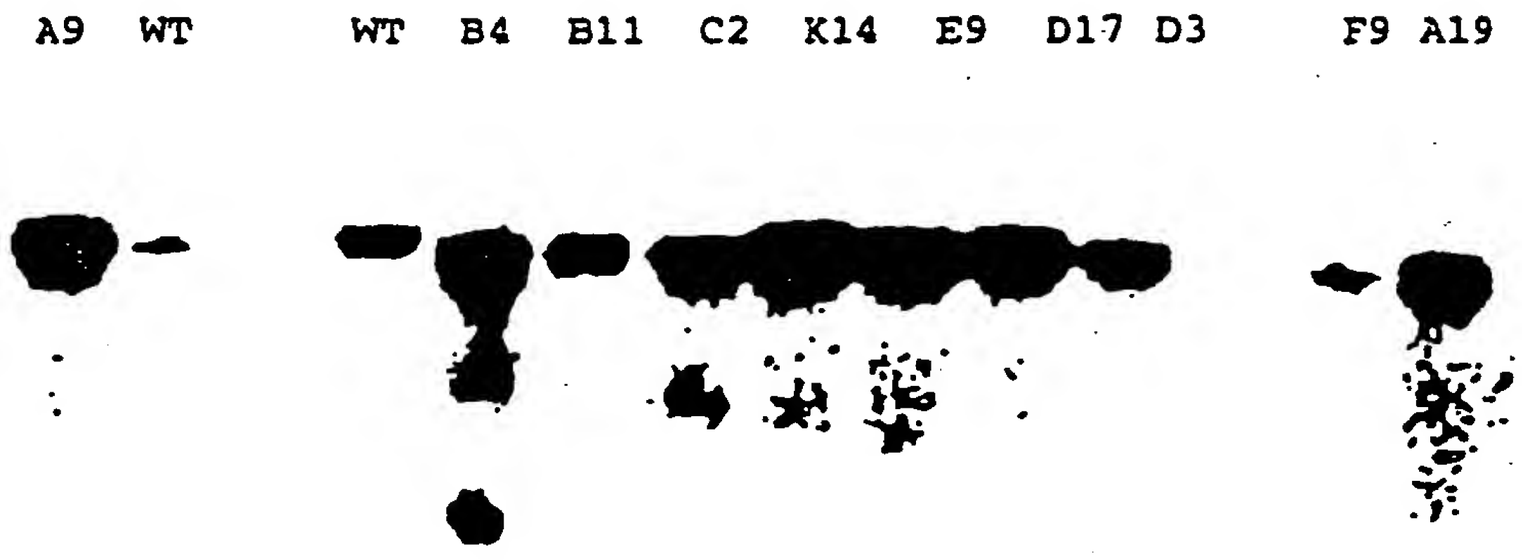
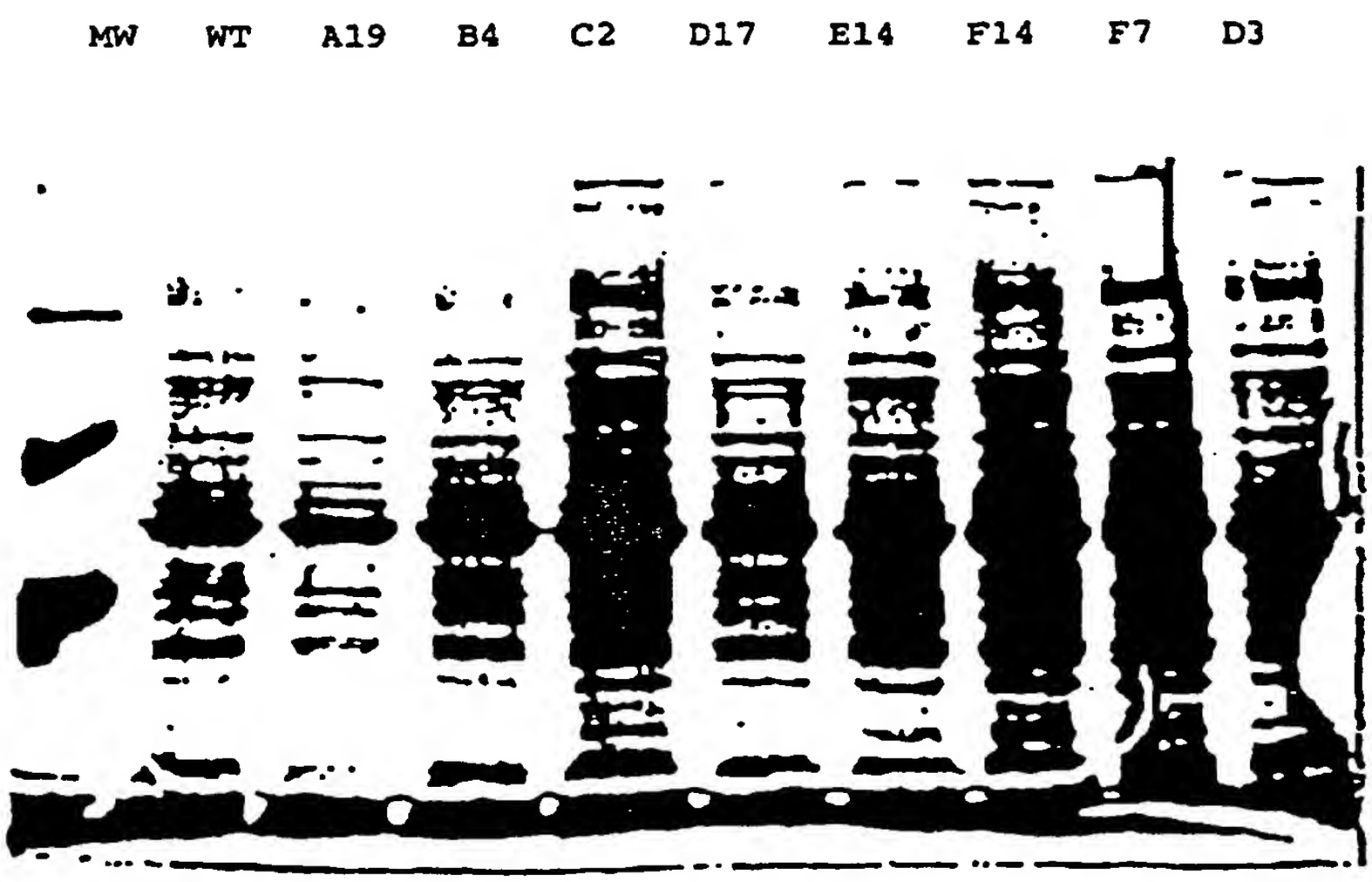
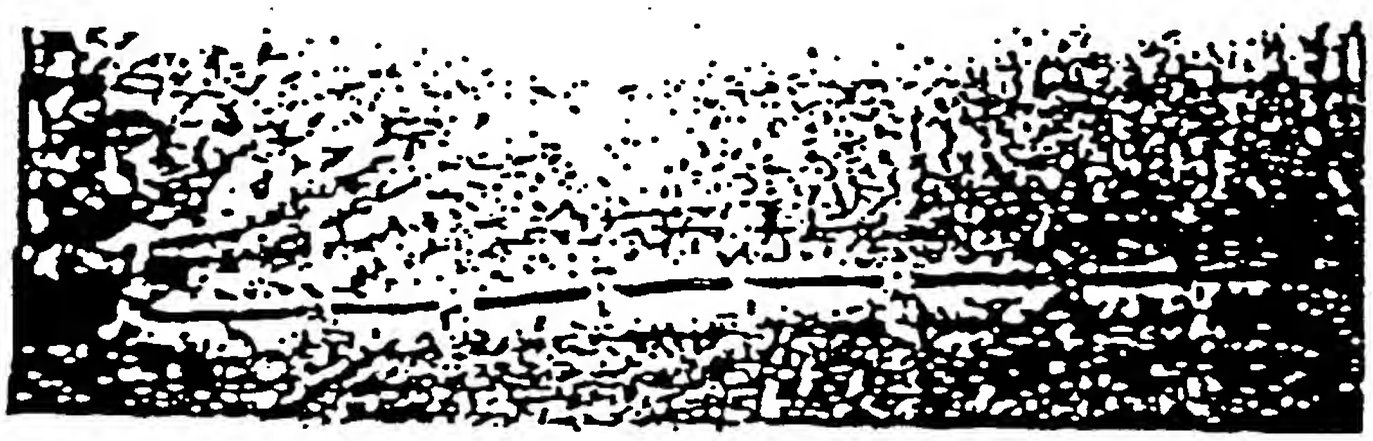


Figure 8: Amounts of protein in transgenic plants



3/NO TAG

Figure 9: Western analysis



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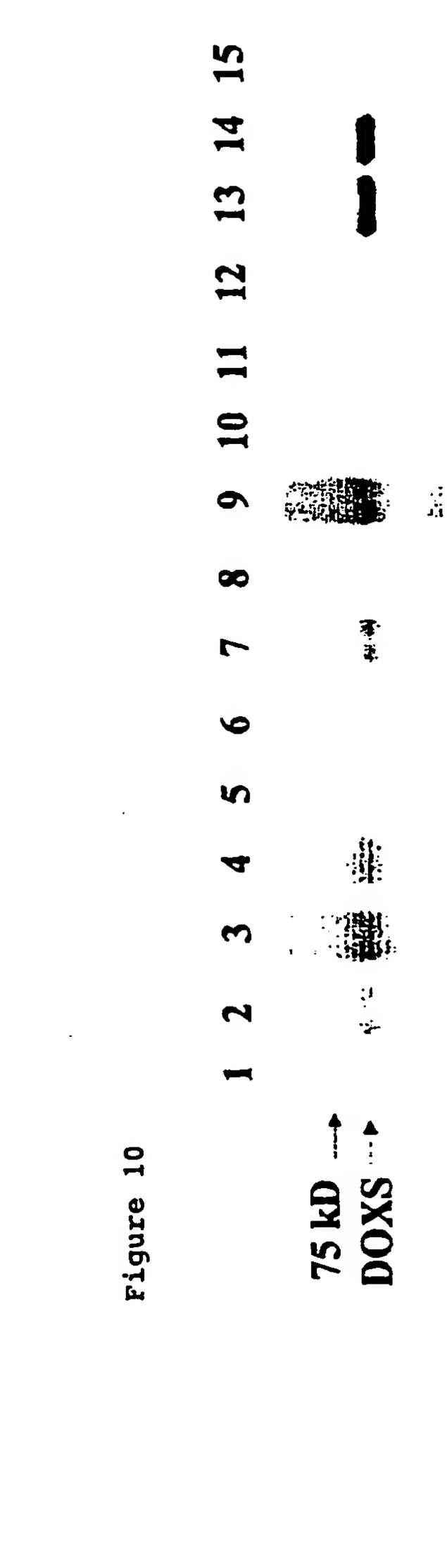


Figure 10

Figure 11

Binary vector for overexpression of the HPPD gene from Streptomyces avermitilis in the cytosol of transgenic plants

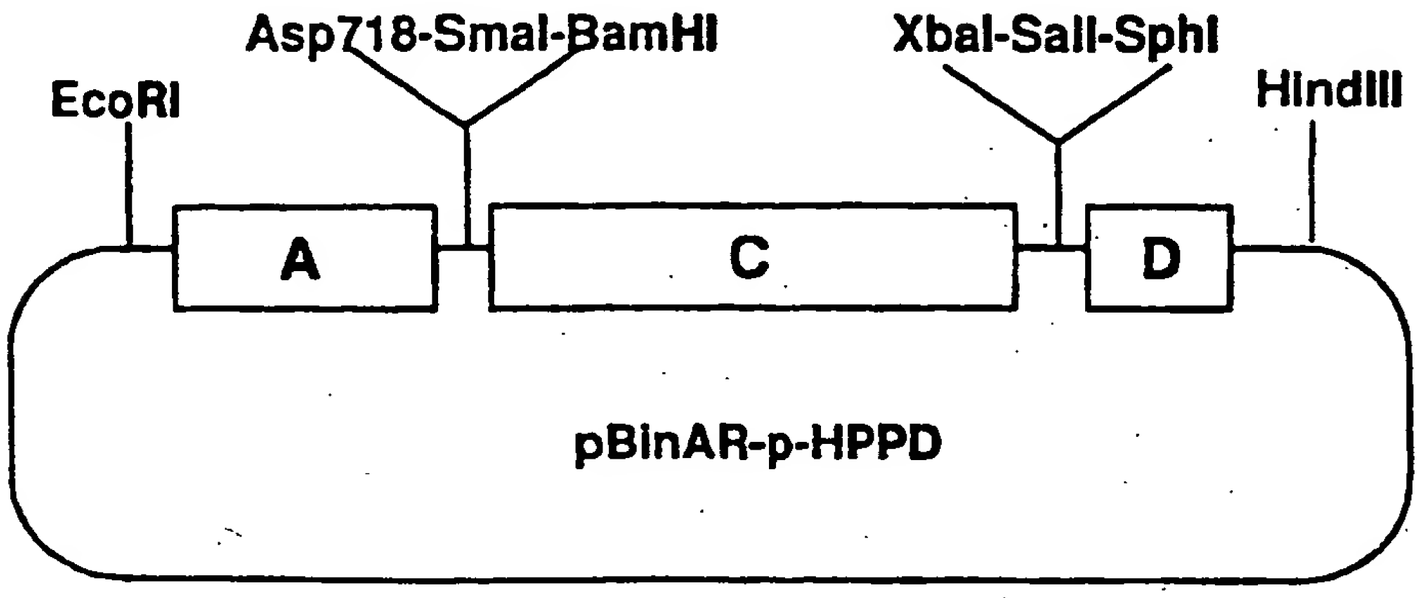


Figure 12

Binary vector for overexpression of the HPPD gene from Streptomyces avermitilis in plastids of transgenic plants

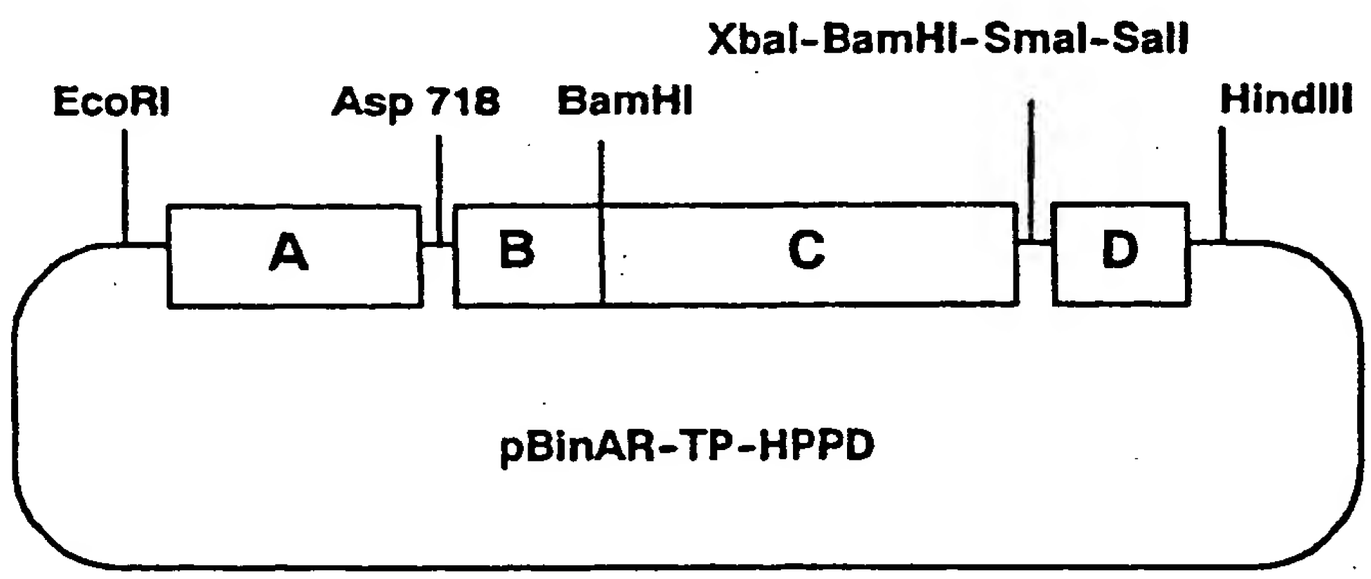


Figure 13

Binary vector for overexpression of the HPPD gene from Streptomyces avermitilis and the DOXS gene from E.coli in plastids of transgenic plants.

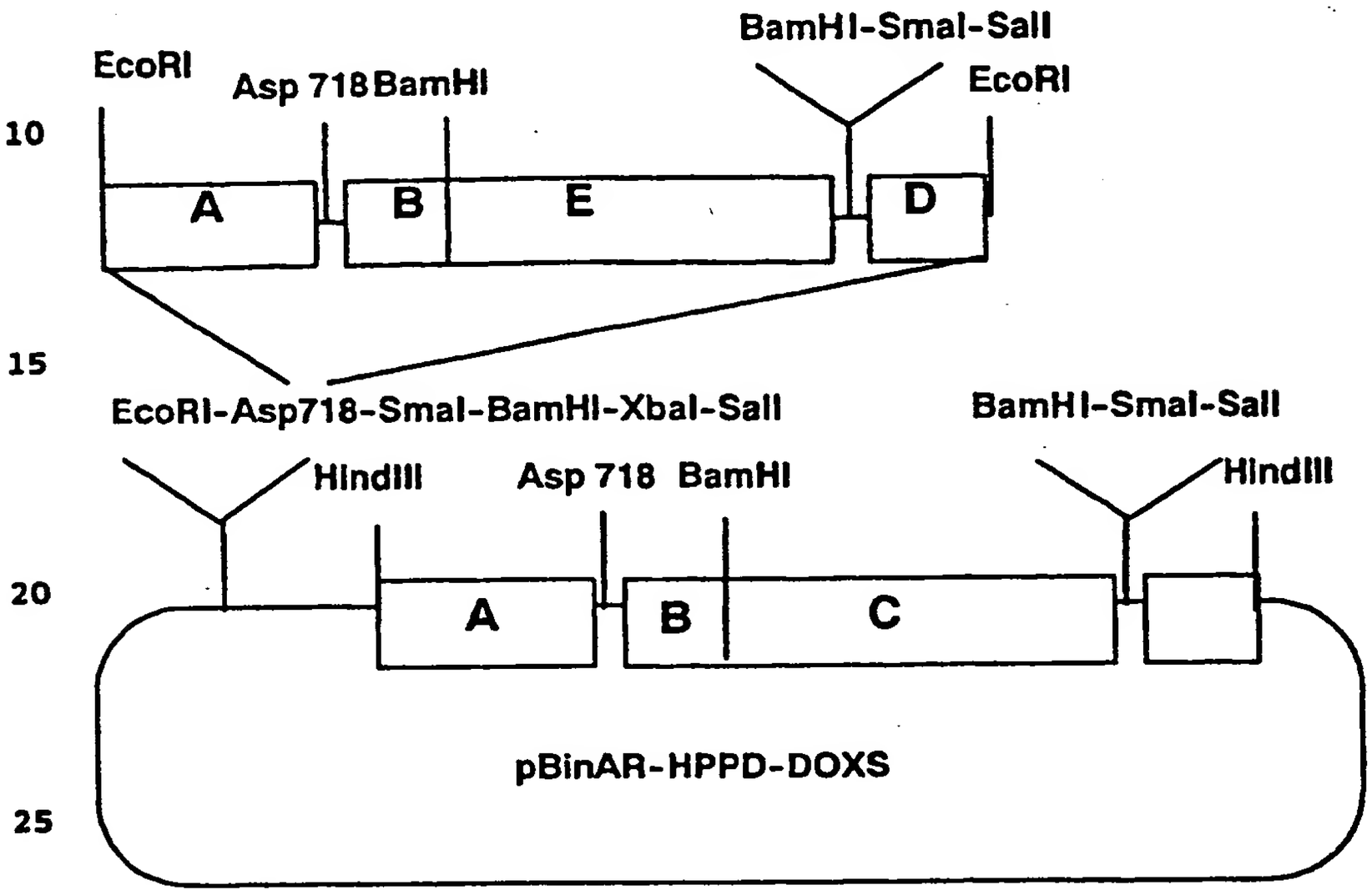
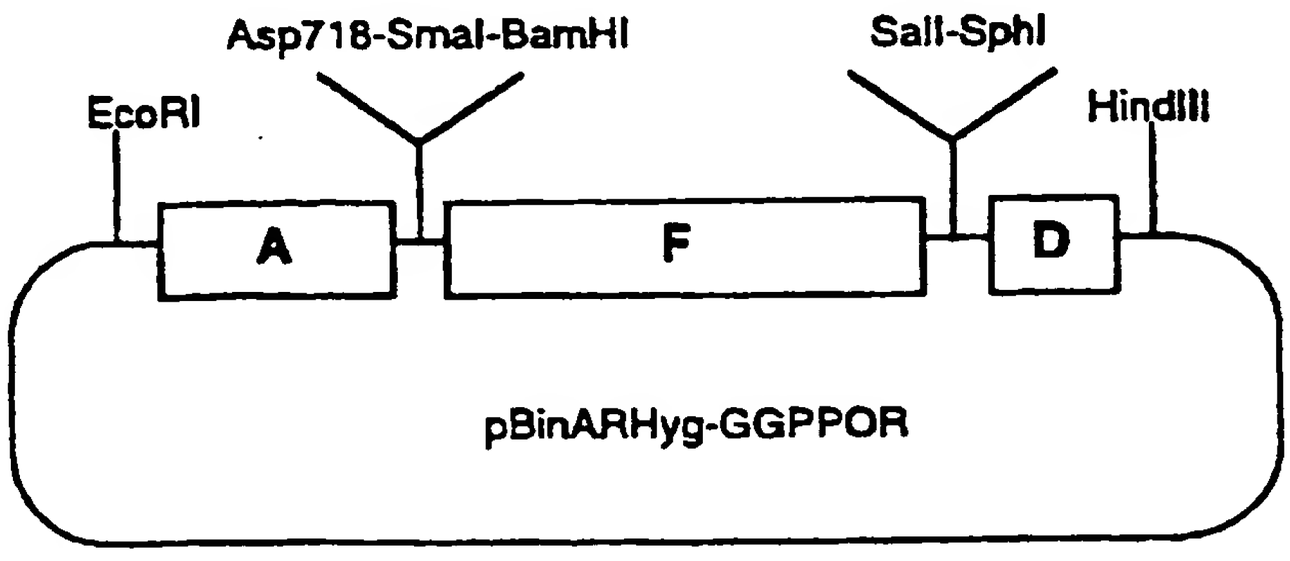


Figure 14

Binary vector for overexpression of the GGPPOR gene from Arabidopsis thaliana in plastids of transgenic plants.



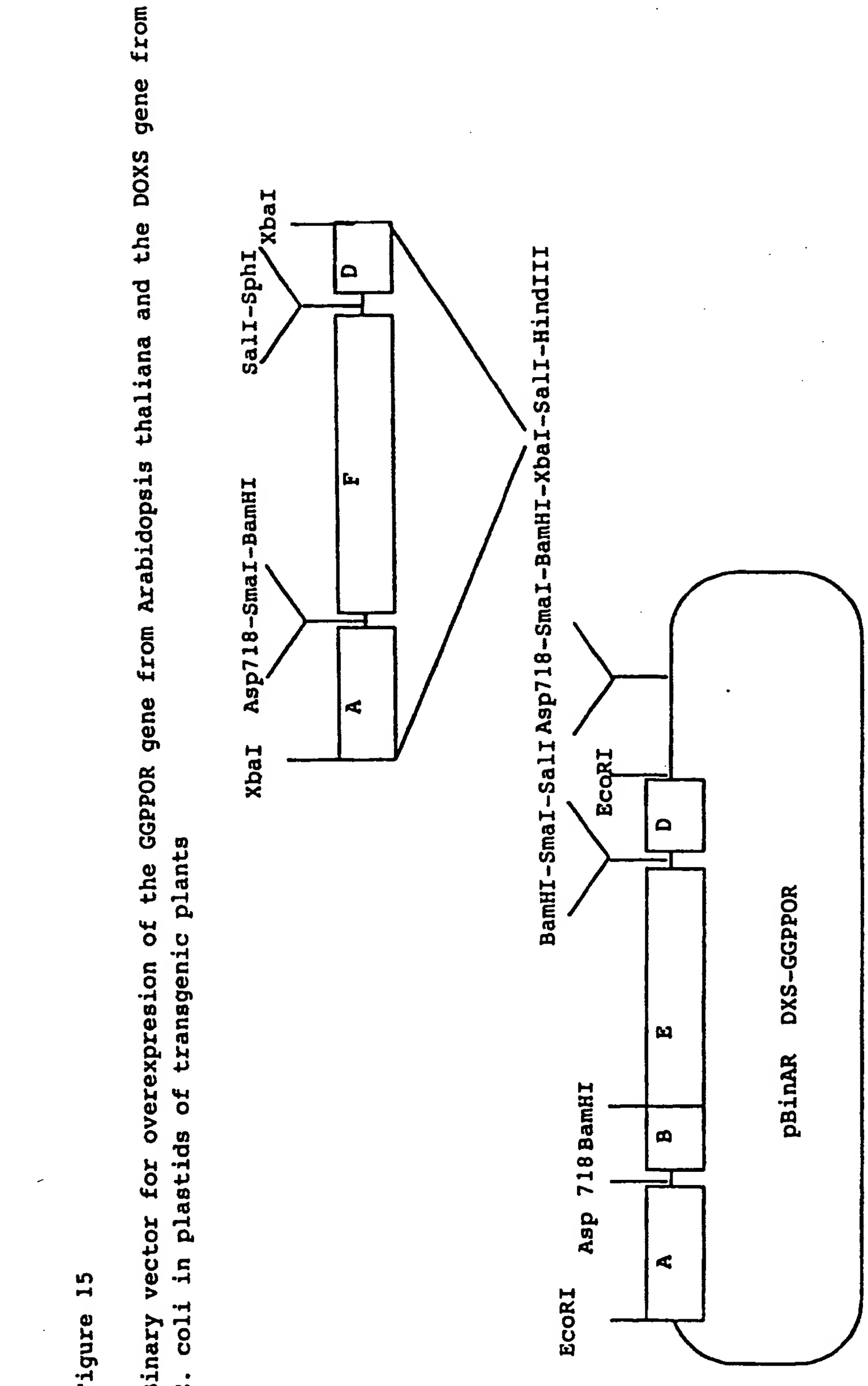


Figure 15

Binary vector for overexpresion of the GGPPOR gene from Arabidopsis thaliana and the DOXS gene from E. coli in plastids of transgenic plants

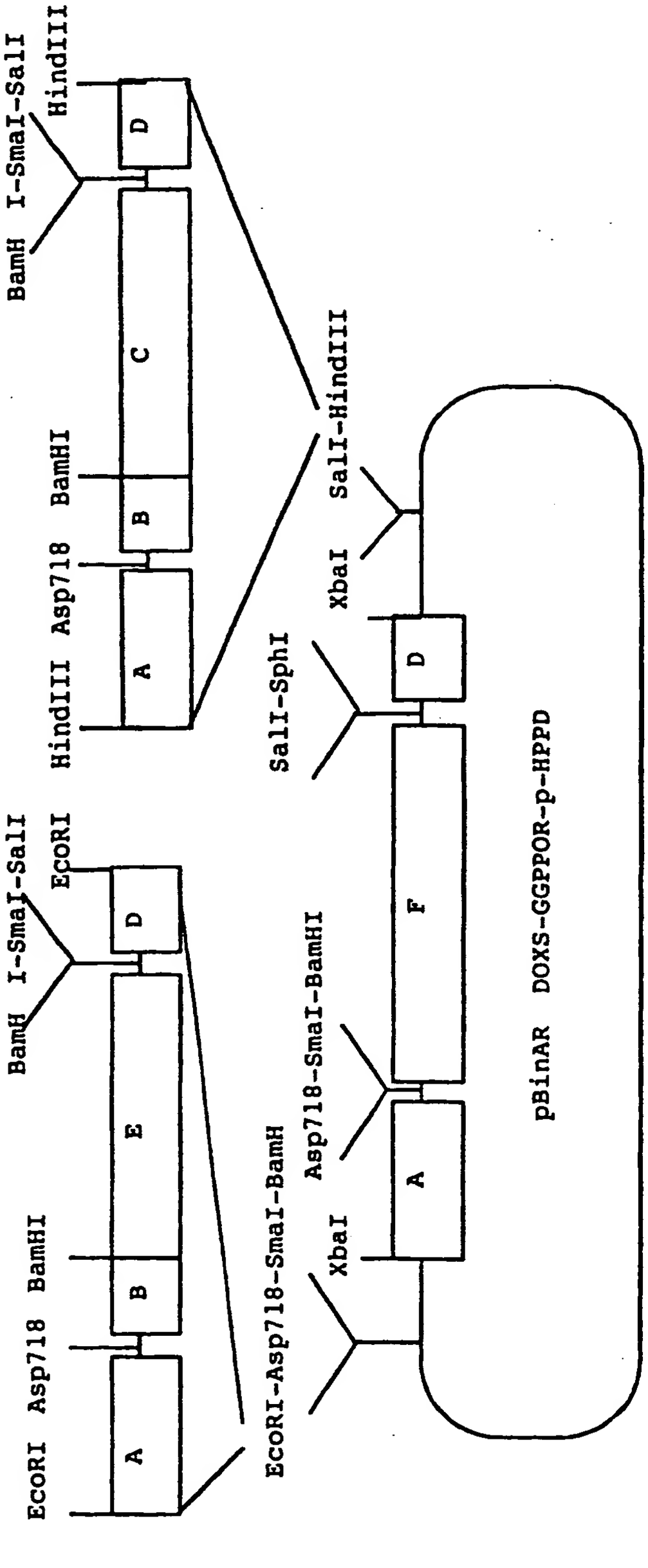


Figure 16

Binary vector for overexpression of the DOXS gene from *E. coli*, the GGPPOR gene from *Streptomyces avermitilis* in the plastids of *thaliana* and the HPPD gen

BINARY VECTOR FOR OVEREXPRESSING THE DOXS-GENE FROM E. COLI, THE GGPPOR GENE FROM ARABIDOPSIS THALIANA AND THE HPPD GENE FROM STREPTOMYCES AVERMITILIS IN THE PLASTIDS OF TRANSGENIC PLANTS